THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII SEPTEMBER, 1946 NUMBER 5

THE FULMINANT FORM OF EPIDEMIC HEPATITIS *

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* Received for publication, May 8, 1946.

Introduction

Epidemic hepatitis has attained pandemic proportions during this war. Large outbreaks have occurred in many parts of the world and in the armies of a number of nations.[1-23] The pathology of this disease as observed in the Army of the United States during the epidemic of 1942 has been dealt with in previous papers.[24, 25] More recently, an acute form of epidemic hepatitis of intense severity terminating fatally in less than 10 days has become prevalent; this we have termed the fulminant form. In a new series of 196 fatal cases occurring between August, 1943, and April, 1945, which we have studied at the Army Institute of Pathology, over half fall into this category. By contrast, in the previous series not a single equally fulminant case was encountered, and only one was reported in the great Swedish epidemic of 1927; [26] the usual duration of the fatal disease was then from 4 to 6 weeks; in other words, the course of fatal hepatitis was predominantly subacute. These divergences in duration reflect striking differences in the pertinent pathologic changes. Thus, in the more fulminant form, the parenchyma of the liver is destroyed completely and uniformly, and this destructive process is accompanied by an intense inflammatory reaction. In the more subacute form seen in 1942 and also in approximately one-fourth of the 1943–1945 series, destruction of the liver is incomplete, the involvement characteristically not uniform, regenerative hyperplasia of surviving parenchyma leads to the production of much new tissue, and inflammation is less pronounced.

Another significant difference is in the epidemiology. In 1942, hepatitis in many instances followed administration of yellow fever vaccine containing human serum. In the new series, such vaccine had not been used; but nearly one-half of the patients had sustained combat trauma. Since seriously wounded patients customarily received transfusion of whole blood, serum, or plasma, it may be assumed that a high proportion of the wounded in this series were thus treated. But it is not known in how many the causal agent of hepatitis was introduced by therapeutic procedures, especially as in several theaters of war large epidemics of hepatitis were prevalent. It is, therefore, an assumption to regard all the wounded cases as examples of "homologous serum hepatitis." This assumption is justified largely by the relatively long interval between date of wound (and presumably of first transfusion) and the clinical manifestation of the disease. The nonwounded cases, comprising approximately one-half of the series, represent both the epidemic and the endemic variants of "naturally" occurring hepatitis.

This study complements the previous report on the pathology of epi-

demic hepatitis [24] and gives a more comprehensive picture of the disease. It is based primarily on 94 fulminant cases in which the clinical duration did not exceed 9 days; however, 39 others with duration of from 10 to 19 days have been used to supply additional information on certain aspects of the disease. This latter group contains many examples indistinguishable from the fulminant form.

The scope and arrangement of this paper are outlined in the table of contents. The methods of study were similar to those employed in the earlier investigations at the Army Institute of Pathology.

Duration of Fatal Hepatitis

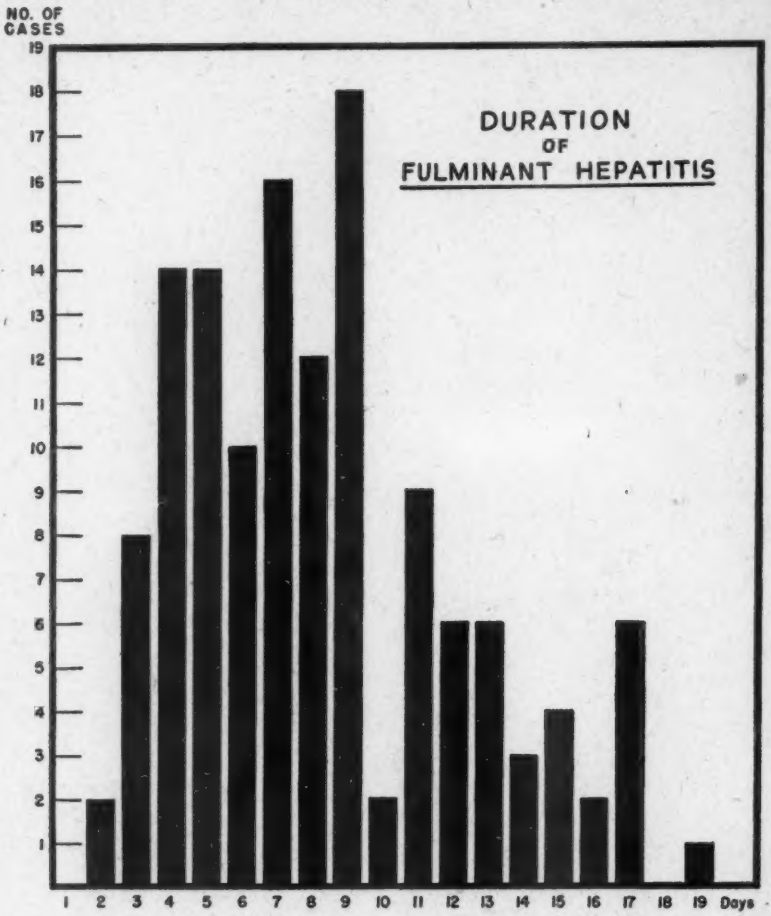
Of the 196 cases studied, the duration of the disease could be determined with fair accuracy in 178. In the remainder the records were inadequate, or the discrepancy between the clinical accounts and the

TABLE I
Duration of Fatal Hepatitis

	Old series: 118 cases (1942 to July, 1943)		New series: 178 cases (August, 1943, to April, 1945)	
Duration	No. of cases	Per cent of cases	No. of cases	Per cent of cases
(*Days*)				
Less than 10	0	0	94	53
10—19	14	11	39	21
20—29	20	16	10	6
30—39	31	26	13	7
40—49	20	17	4	2
50—59	8	7	2	1
60—69	8	7	1	0.5
70—79	9	8	3	2
80—89	0	0	1	0.5
90—100	5	5	3	2
Over 100	3	3	8	4

character of the lesions was so great that this group was excluded from analysis. The pertinent data for the series are given in Table I. It will be noted that in 53 per cent the course of the disease, from beginning of symptoms to death, was less than 10 days, and that in 74 per cent the disease terminated fatally within 20 days. A more detailed analysis of this group is shown graphically on a day-by-day basis in Text-Figure 1, where the vertical columns represent the number of cases and the abscissae the corresponding duration of disease. Inspection of the graph brings out, first, that the number of cases in which death occurred within so short a time as 4 days is considerable (21 per cent); second, that the number of fatalities on any one day between the fourth and the ninth is greater than the number for any subsequent day; and third, that there is a sharp drop in the number of deaths beyond the

ninth day which marks a turning point. This impression is strengthened by the character of the lesions: in almost all of the cases which ended fatally before the tenth day, destruction of liver cells was complete or nearly so, whereas in those with a longer survival period, de-



Text-Fig. 1. Duration of the disease in 133 cases of fulminant hepatitis.

struction usually was incomplete. It is for this reason that we have considered the cases terminating in less than 10 days as representing a fairly homogeneous type, namely, the fulminant form of hepatitis.

Comparison of the duration in the present series with that of the epidemics of 1942 in the United States Army, and of 1927 in the civilian population of Sweden brings out marked differences which have al-

ready been indicated. Suffice it to state that the median duration in the present series is 8 days, whereas in the other epidemics it exceeded 5 weeks.

The search for the elements responsible for the more fulminant course of hepatitis during the past 2 years is a cardinal task of this investigation.

Incidence of Previous Trauma and Transfusion

Factors that can clearly be correlated with duration of disease are previous trauma and subsequent transfusions of blood (Table II). In nearly all cases trauma—combat wounds or burns—was of serious

TABLE II

Incidence of Previous Trauma (Wound or Burn) in 178 Cases of Fatal Hepatitis (New Series)

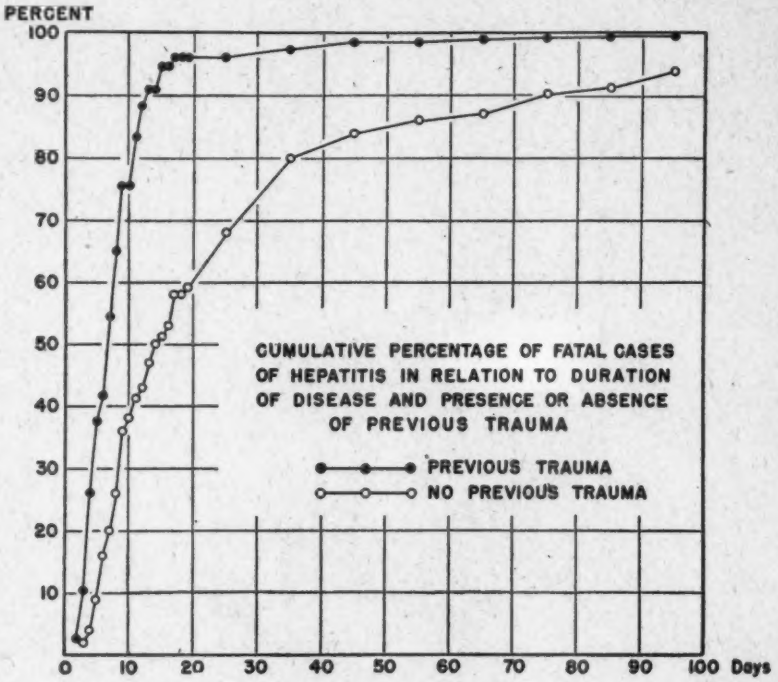
Duration of disease	Wounded	Burned	No previous trauma	Total number of cases
(*Days*)				
Less than 10	52 (55%)	6 (6%)	36 (38%)	94
10—19	16 (41%)	0	23 (59%)	39
20 and over	3 (7%)	0	42 (93%)	45
Total no. of cases	71 (40%)	6 (3%)	101 (57%)	178

degree and was sustained within 4 months of the onset of hepatitis. Most of the wounded received transfusions of blood or its derivatives within a few days of injury: hence these cases probably may be regarded as examples of homologous serum hepatitis. It is from this premise that we here analyze the relation of trauma to the duration of fatal hepatitis.

Consideration of Table II reveals the significant fact that the patients with hepatitis who had previously sustained trauma succumbed much sooner than those who had not been injured. Thus, while there was history of trauma in 61 per cent of the total group in which death occurred within 10 days, the incidence dropped to 41 per cent for the group with a duration of from 10 to 19 days, and to only 7 per cent when survival exceeded 20 days. Conversely, column 4 of the table shows a progressive increase in nontraumatic cases in proportion to duration. These variations are graphically represented in Text-Figure 2, where the cumulative percentage of fatal cases has been plotted in relation to duration of disease and presence or absence of previous trauma. Here it is shown that 76 per cent of those cases with a history of trauma ended fatally within 10 days of the onset of symptoms, and

96 per cent by the 20th day, whereas the corresponding percentages for the nontraumatic group are 38 and 59.

Computation of the median duration of hepatitis in the two groups gives 7 days for the wounded and 15 days, or twice as long, for the group without trauma. The contrast between the two groups is shown graphically on a day-by-day basis in Text-Figure 3 using the same



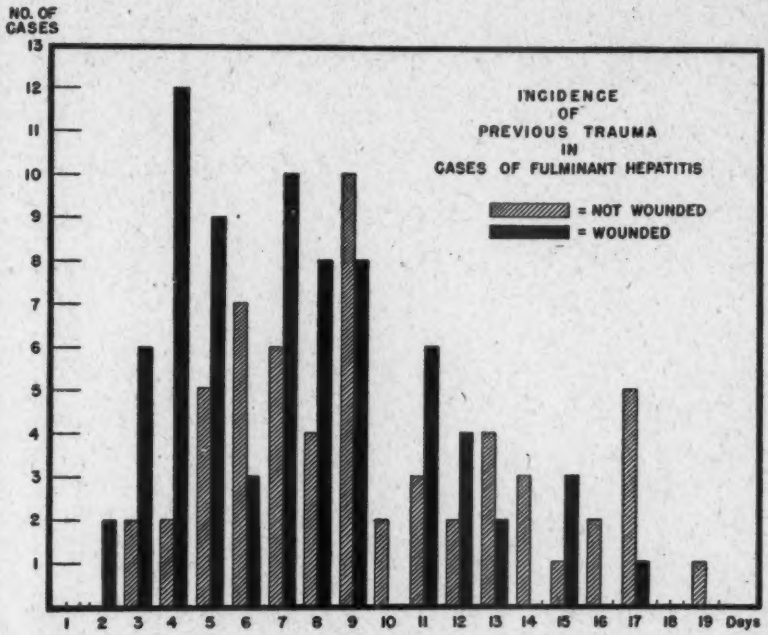
Text-Figure 2

data from which Text-Figure 2 was constructed. The graphs show that the course in the wounded group tends to be more fulminant than in the nonwounded; the mortality of patients with a history of trauma and transfusion was five times as great during the first 4 days after onset of symptoms. Evidence favors the conclusion that the type of fatal hepatitis which may follow trauma and transfusions of blood, *i.e.*, homologous serum hepatitis, tends to run a considerably more rapid course than does the naturally occurring disease.

Distribution of Cases According to Age and Race

The data from 130 cases of hepatitis in which the clinical course was less than 20 days are given in Table III. All were male soldiers (in-

cluding 7 Italian and German prisoners of war); 125 were white, 3 Negroes, one a Javanese, and one an American-born Japanese. The distribution is similar to that in the 1942 epidemic, and to that in a recent survey of 1,762 patients under treatment for hepatitis in Army General Hospitals.[27] It is almost exactly in direct ratio to the population in the Army. Factors of age, sex, or race, therefore, do not ac-



Text-Fig. 3. Comparative mortality on a day-by-day basis between nonwounded and wounded patients with fulminant hepatitis.

count for the preponderance of a more fulminant form of hepatitis in the present series.

TABLE III

Age in 130 Cases of Hepatitis in Which the Duration of the Disease Did Not Exceed 19 Days

Years	No. of cases	Per cent of cases
Less than 20	10	8
20—24	45	35
25—29	41	32
30—34	25	18
35—39	6	5
Over 40	3	2

Geographic Distribution and Epidemiologic Types of Fatal Hepatitis

In order to determine possible variations of the disease in relation to climate and other factors, the cases in the present series have been tabulated according to geographic location and duration of disease, and subdivided as wounded and nonwounded groups in each theater of war (Table IV). In this tabulation the European theater includes Great Britain and the continent except the Italian peninsula; the Mediterranean theater includes Italy, Africa, and the Middle East; the Pacific theaters include the islands of the Pacific, and India, China, and Burma. It will be noted that 90 per cent of the cases came from combat areas and only 10 per cent from the United States.

TABLE IV

Geographic Distribution of 178 Fatal Cases of Hepatitis (New Series)

Duration of disease	United States		European theater		Mediterranean theater		Pacific theaters	
	Wounded	Not wounded	Wounded	Not wounded	Wounded	Not wounded	Wounded	Not wounded
(*Days*)								
Less than 10	2	5	31	2	20	21	5	8
10—19	0	4	11	5	2	6	3	8
20 and over	0	7	1	6	2	8	0	21
Total	2	16	43	13	24	35	8	37

During the period covered by this study, extensive epidemics of hepatitis existed in the Mediterranean and Pacific areas, and were absent (except during the final months) in the European theater and in the United States. Cases in the first two areas may, therefore, be regarded as belonging predominantly to the epidemic type; in the latter areas, to the endemic or sporadic type.

In the wounded group each casualty has been credited to the theater in which the injury occurred, although the first symptoms of hepatitis often did not develop until after evacuation to the United States.

The cases in the series may, with certain assumptions, be classified on the basis of epidemiology. There were 29 examples of the endemic and 72 of the epidemic type of the "naturally" occurring disease, and 77 of homologous serum hepatitis, most of the latter coming from the European theater. Cases without trauma predominated slightly in those from the Mediterranean theater and strikingly so in those from the Pacific.

Mortality

In all recorded epidemics of hepatitis the mortality has been low, ranging from 0.2 to 0.4 per cent. Thus, during the United States Army epidemic of 1942 there were 51,337 cases reported,[11] with a mortality rate of 0.24 per cent. During the more recent Army epidemics, 68,000

cases (in round numbers) and 196 deaths have been recorded; that is to say, the death rate was 0.3 per cent. In view of the change in character of fatal hepatitis during recent years the agreement in mortality rates is surprising.

It should be emphasized, however, that the rate for the 1943–45 epidemics is based upon incomplete returns and must be regarded as an approximation. Actually, the mortality of "naturally" occurring hepatitis is probably lower, whereas that of homologous serum hepatitis may be higher. No precise information is as yet obtainable on either variant. Estimates are based largely upon cases which have been hospitalized; no account usually is taken of the mild and frequently nonicteric forms of the disease which escape recognition. For homologous serum hepatitis, attempts to determine case mortality have been particularly unsatisfactory, but the impression is that it may be several times as high as in the naturally occurring disease. The mortality of 0.3 per cent in the epidemics under discussion is an average, and has varied in different localities and at different times.

With regard to duration of the endemic type, it is interesting to note that there were 16 acute and 13 subacute fatal cases (defining the latter as running a course of more than 20 days); they were almost equally divided between the United States and Europe. In the epidemic group, subacute cases slightly outnumbered acute cases in the Pacific theater (21 to 16), whereas in the Mediterranean area the proportions were more than reversed (8 to 27).

No correlation was apparent between duration of illness and geographic location in the endemic type. For the epidemic type, however, the disease was predominantly acute in the Mediterranean and subacute in the Pacific areas. Measured by duration it appears to have been more virulent in the Mediterranean than in the Pacific areas.

In the homologous serum group, practically all cases ran an acute course without any apparent influence by geographic factors; only 3 were subacute. It would be a mistake, however, to assume at once that this is a general characteristic of this type of hepatitis.

Clinical Picture of Fulminant Hepatitis

A number of recent reports by American,[11–18] British,[4–10] and German[19–23] observers have dealt with the clinical picture of epidemic hepatitis in its usual benign form ending in recovery. Wherever the disease has occurred, the clinical pattern has been remarkably uniform; the symptoms and signs have varied in degree, not in kind.[28] There is an impression, however, that in recent years hepatitis has become more severe.

In this paper we are concerned with a fatal form in which nearly all

liver cells are destroyed within a few days. In an effort to correlate lesions and functional disturbances an analysis has been made of the clinical manifestations of the fulminant cases. The analysis is based primarily on the 94 cases of that group, but other cases are incorporated in certain sections since their clinical and anatomic manifestations were identical even though survival was longer (10 to 19 days). The general data have been augmented by abstracts of the clinical records of representative cases.

Similarity between "Spontaneous" and "Inoculation" Hepatitis

A question of great importance arose in connection with the analysis: Is "naturally" occurring fulminant hepatitis, as seen in either its endemic or epidemic variants, clinically distinguishable from the disease "artificially" induced by parenteral injection of blood or its derivatives? The answer can readily be given: Except for a tendency to a more rapid course in serum hepatitis, no differences were discernible. This finding is in accord with observations on nonfatal cases.[3,9,19,20,29–33] It is, therefore, permissible to combine the two groups for purposes of analysis.

Course of Fulminant Hepatitis

The three phases previously reported in the subacute form can usually be recognized in the fulminant disease: (1) an initial, prodromal or pre-icteric stage; (2) an intermediate stage which begins with the onset of jaundice, and (3) a final stage which generally is ushered in abruptly by grave nervous manifestations. The time relations were less sharply defined in the fulminant than in more protracted cases. The pre-icteric period varied greatly (Text-Fig. 4) but was generally 2 or 3 days. The duration of the terminal phase did not differ significantly from that of the previous series, ranging from 1 to 4 days. Since the total duration in the fulminant group was less than 10 days, it is evident that the intermediate stage was often very brief; indeed, in some instances the manifestations of the prodromal and final stages merged.

Initial Symptoms. Two usual combinations of initial symptoms may be recognized: (1) An "infectious" type in which the disease is ushered in suddenly by the symptoms common to many acute infections: fever, chilliness, or, more infrequently, shaking chills, malaise, generalized aching, pains in the joints, back, and eyes, with gastrointestinal symptoms usually following in a day or so but rarely dominating the picture. (2) A "gastrointestinal" type in which the initial symptoms are similar to those encountered in the subacute variety of the disease: anorexia, nausea, epigastric discomfort or pain, with variable elevation in tem-

perature. The onset usually is more gradual with the second than with the first group; but it may be acute with severe nausea and vomiting from the start.

Sometimes initial symptoms of both types appear simultaneously, with neither in the foreground. Rarely, the disease begins with mental disturbances. The distribution of these several types of onset is shown in Table V which is based upon the fulminant cases and 19 others of more than 9 days' duration but with similar manifestations. It will be seen that four-fifths of the cases belong to the two main groups, which, roughly, are represented equally.

The 3 cases in which cerebral symptoms ushered in the disease deserve special mention. An indication of the extent to which central

TABLE V

Predominant Types of Initial Symptoms in 113 Cases of Hepatitis in Which the Duration Did Not Exceed 19 Days

Symptoms	No. of cases	Per cent of cases
Gastrointestinal type	48	43
Acute infectious type	40	36
Mixed gastrointestinal and acute infectious type	22	19
Mental disturbances	3	3

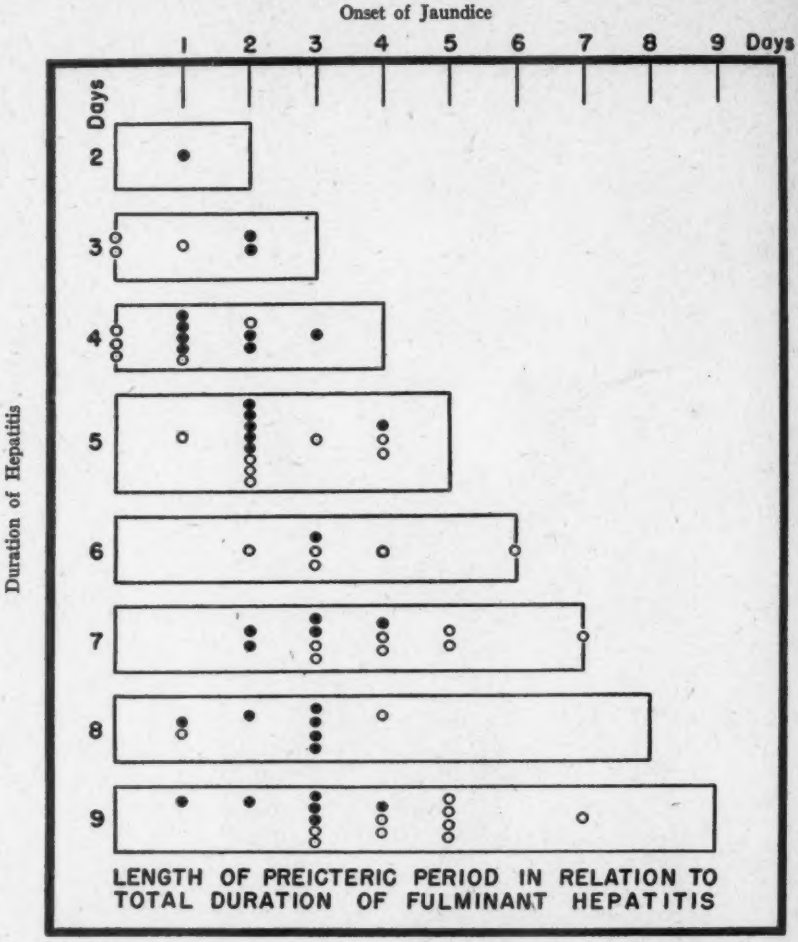
nervous manifestations may dominate the clinical picture is afforded by the fact that exploratory craniotomy was performed in 2 of these cases.

The prodromal symptoms observed in fulminant cases do not differ significantly in type or severity from those of the usual nonfatal cases. The predominant initial symptoms of the fulminant cases, as of those with recovery, have been independent of locality, and those of the two main types have coexisted during the same periods of epidemics although their relative frequency has varied. Thus, in an American Army Hospital in Italy, the "infectious" type occurred in 61 per cent, the "gastrointestinal" form in 39 per cent; [18] in a British garrison at Malta the proportions were reversed, only 37 per cent exhibiting the febrile type; [6] during an epidemic in the Middle East the two were represented equally.[34] The symptoms and signs of the febrile type have been described as closely resembling malaria,[19,36] influenza,[2,35] sand fly fever,[6,8] and "acute surgical abdomen." [4]

The observation that the subsequent clinical manifestations bear no relation to the character of initial symptoms is true for the fatal as well as for the nonfatal form of hepatitis.[34]

Onset of Jaundice. In Text-Figure 4 is shown the relation between

the onset of jaundice and the duration of disease in 72 fulminant cases. Those previously wounded (34 in number) are represented by solid circles; the nonwounded (38 in number), by open circles. The graph brings out several points of interest. First, it shows no significant dif-



Rectangles = duration of hepatitis; solid circles = onset of jaundice in wounded cases; open circles = onset of jaundice in nonwounded cases.

Text-Figure 4

ference in time of appearance of jaundice in wounded and nonwounded cases; in both the "spontaneous" and the "inoculation" variants there is considerable scattering. In several instances jaundice occurred at the onset of symptoms; in others it was delayed until the day of death; but on an average it appeared on the second or third day after the be-

ginning of symptoms. A positive correlation between onset of jaundice and duration of disease seems to be indicated by early jaundice in the more fulminant cases.

The time of appearance of jaundice and its subsequent intensity bore no relation to the type of initial symptoms. This same lack of correlation has been reported in nonfatal cases.[18,36]

Final Stage. The main features of the terminal phase were not appreciably different from those described in the earlier series. Usually a rather abrupt change for the worse was initiated by cerebral symptoms beginning with listlessness, drowsiness, increasing apathy; with restlessness, incoherence, disorientation; or with irritability and failure to cooperate. The symptoms of excitement often progressed to an acute maniacal state necessitating restraint. Frequently apathy and excitement alternated, but in the majority of cases deep coma eventually supervened. With the onset of cerebral symptoms hyperactivity of certain reflexes was the rule; a positive Babinski sign and persistent ankle clonus were common. Muscular twitching was frequent, while opisthotonos and generalized convulsions were occasional.

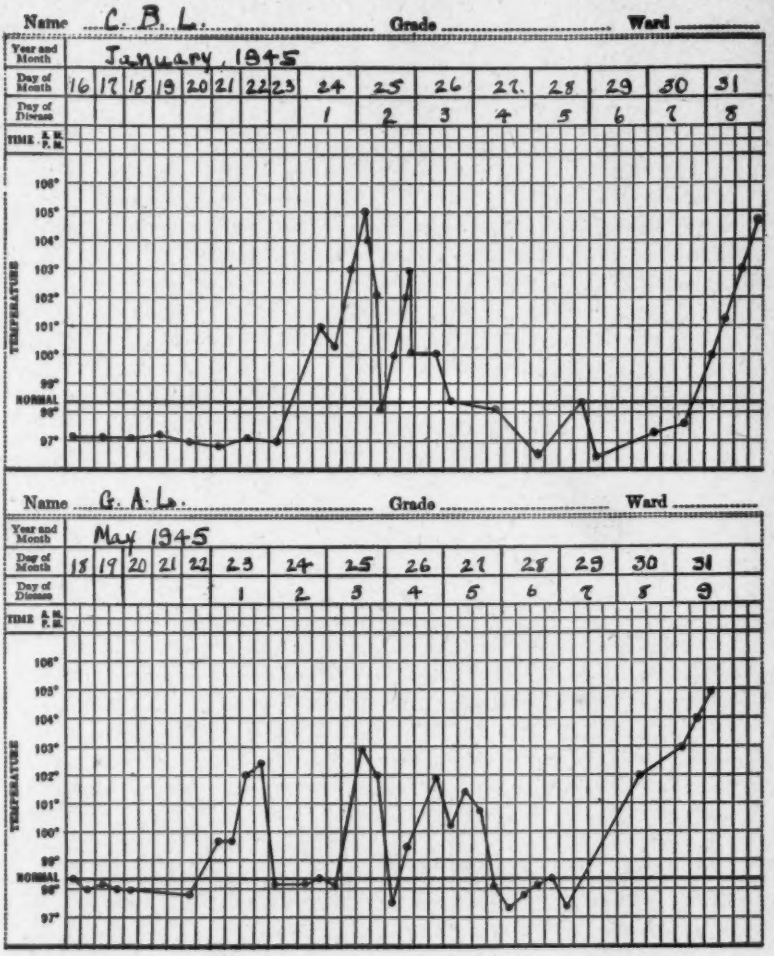
During the final phase jaundice often deepened rapidly. Vomiting frequently was severe and sometimes projectile. Bleeding from the gastrointestinal mucosa was evidenced by coffee-ground vomitus and melena, and was occasionally associated with purpuric phenomena or with frank gastrointestinal hemorrhage of considerable magnitude. Signs of pulmonary edema or patches of consolidation were found in the majority. Shock developed in a number of cases; in all there was a terminal rise of temperature.

Symptoms, Signs, and Laboratory Findings

The analysis of the data included the following features: temperature, pulse rate, palpation of liver and spleen, recurrence of disease, jaundice, icterus index, total and differential leukocyte count, plasma proteins, urine, nonprotein nitrogen and urea nitrogen of blood, and blood sugar. Abstracts of representative clinical records illustrate these data.

Temperature. Temperature records for the prodromal stage were available in 68 fulminant cases; in all but one fever ranged from 99° to 104° F. and averaged 102° F. The peak usually was attained on the second day. With the onset of jaundice the temperature, as a rule, fell to normal or slightly below normal (96° to 97° F.) and remained so for several days, but in about one-fourth of the cases irregular subsequent rises took place. During the final phase the temperature almost invariably rose sharply, usually to 103° to 105° F., occasionally

to as high as 107°. Representative temperature curves are given in Text-Figure 5. Except for the terminal fever, the temperature reactions in fulminant hepatitis are similar to those in nonfatal cases;

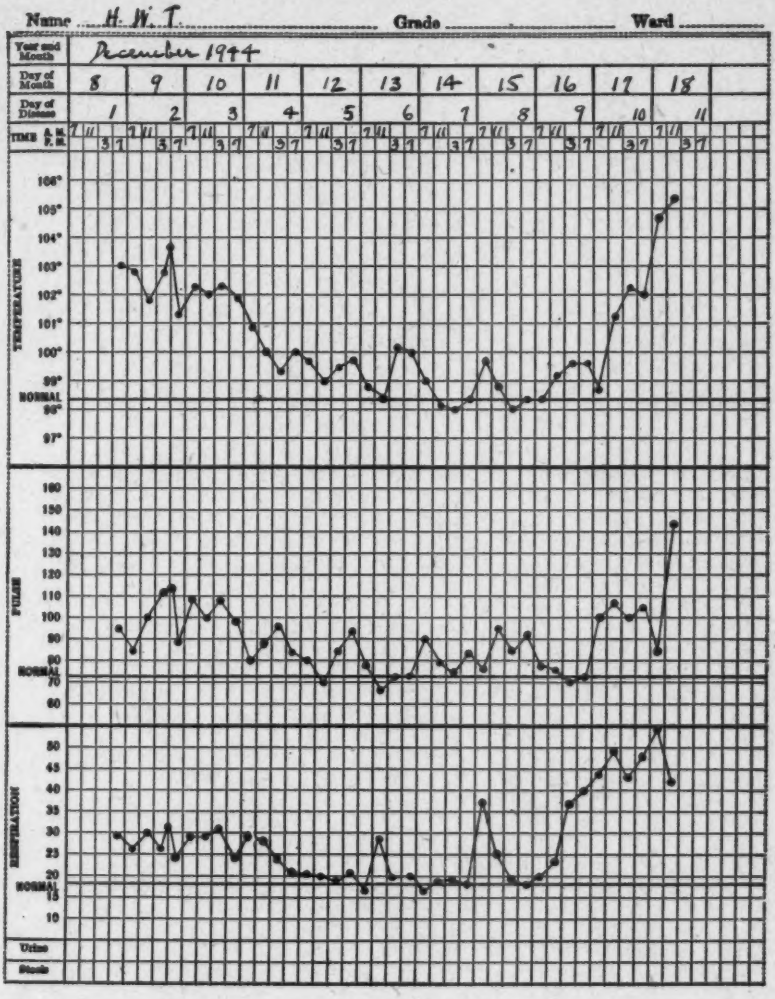

Name C. B. L. Grade Ward
Year and Month January, 1945
Day of Month 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Day of Disease 1 2 3 4 5 6 7 8
TIME A.M. P.M.
TEMPERATURE
106° 105° 104° 103° 102° 101° 100° 99° NORMAL 98° 97°
Name G. A. L. Grade Ward
Year and Month May 1945
Day of Month 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Day of Disease 1 2 3 4 5 6 7 8 9
TIME A.M. P.M.
TEMPERATURE
106° 105° 104° 103° 102° 101° 100° 99° NORMAL 98° 97°

Text-Fig. 5. Representative temperature curves in cases of fulminant hepatitis.

most observers have reported a fever which was often high at the onset [2,4,6,8,9,16,18,19,34,36,37] and usually declined as jaundice appeared, although sometimes it was protracted.[8,10,34,37]

Pulse Rate. The pulse rate was nearly always elevated during febrile periods (Text-Fig. 6). Bradycardia was observed only in rare instances.

Palpation of Liver and Spleen. The liver was moderately enlarged and usually tender during the late prodromal and early icteric stages; as the disease progressed shrinkage was frequent but not invariable.



Text-Fig. 6. Clinical chart of a case of fulminant hepatitis with death on the eleventh day. Pulse rate rose during periods of fever.

Enlargement of the spleen was less constant; it was recorded throughout all stages in approximately 25 per cent of the cases. More precise statements concerning the sizes of these organs have little meaning because of the variability at different stages of the disease. In nonfatal

cases enlargement of the liver has been reported invariably when the disease was present in moderate or severe form, and frequently when it was mild; [37] other writers have recorded enlargement in the majority.[8,13,16,18,19,34]

For the spleen the figures usually given are 10 per cent or less; [13,14,16,18,34] although some observers have found readily palpable spleens more frequently, *i.e.*, in 13.6 per cent,[37] in 27 per cent,[8] or "usually." [22]

Recurrence. It is generally thought that a high degree of immunity is conferred by an attack of epidemic hepatitis, although accurate information is not available.[33] When second attacks do occur they are perhaps induced by nonspecific factors, by immunologically different strains, or by large doses of the infectious agent.[5]

TABLE VI

Icterus Index in 60 Cases of Fulminant Hepatitis

Icterus index	No. of cases	Per cent of cases
Below 50	9	15
50 — 99	31	53
100—149	15	24
150—over	5	8

In the present series 4 patients had a history of a previous attack within 3 years of the fatal disease; 1 within 3 months, the remainder within 2 to 3 years. It is interesting to note that the livers of these patients presented no scarring to indicate previous injury; this observation is in harmony with our earlier observations.[25]

Jaundice. In contrast to the predominant subacute form of the 1942 epidemic, icterus was light or moderate, and rarely deep even during the terminal stage. At least 2 of the fulminant cases remained completely free from jaundice, and in a third only the scleras were faintly tinged. The clinical abstracts of these anicteric cases are included among those chosen to give a representative picture of the disease as seen in individual cases.

Icterus Index. The values of the index usually conformed with the relatively light icterus. The data for 60 cases, based upon the highest values recorded, are given in Table VI. The index in approximately two-thirds of the series did not exceed 100. Values above 150 were found in only 8 per cent.

Total and Differential Leukocyte Count. During the prodromal period a tendency toward leukopenia was evident in 20 of 27 cases

(Table VII). The differential count disclosed a slight lymphocytosis, *i.e.*, 9 of 10 patients had a lymphocyte-monocyte count between 40 and 49 per cent. In the icteric phase total counts exceeded 11,000 in approximately one-half, the elevation probably resulting from pulmonary complications or hemorrhages. A slight neutrocytosis was also observed during this phase.

Plasma Proteins. Determinations made in 25 cases were slightly or definitely below the normal in almost every instance (Table VIII).

TABLE VII

Leukocyte Count in Cases of Fulminant Hepatitis

Leukocyte count	Pre-icteric period	Icteric period
Below—5000	7	0
5000 —6900	13	7
7000 —8900	4	7
9000 —10,900	0	8
11,000—12,900	2	10
13,000—14,900	0	4
15,000 and over	1	14
Total no. of cases	27	50

Because of therapeutic transfusions of blood or plasma, the value of these figures is questionable; without treatment much lower levels might have been obtained.

Urine. The urine was usually normal in the first day or two of illness, but as a rule by the third or fourth day changes were noted. The specific gravity was moderately elevated, bile was almost invariably present, albumin of slight degree appeared in two-thirds, and casts in approximately one-half. The casts were sometimes hyaline, sometimes granular, and frequently of both types. Leucine and tyrosine crystals were frequently searched for, but very rarely found.

TABLE VIII

Serum Proteins in 25 Cases of Fulminant Hepatitis

Below 5.0 gm./100 cc.	3
5.0 5.9 gm./100 cc.	5
6.0 6.9 gm./100 cc.	9
7.0 and over	8

Nonprotein Nitrogen; Blood Urea Nitrogen. Of 31 representative cases selected solely on the basis of completeness of records, the nonprotein nitrogen level of the blood fell within the normal range of 25 to 40 mg. per cent in 16 cases (Table IX). In 15 cases there was evidence of nitrogen retention. In 11 of these, the values ranged between 41 and 60 mg. per cent; in 4 cases they were above 70 mg. per cent.

Blood urea nitrogen was also determined in 10 instances. In 2 the values were 16 and 16.6 mg. per cent, respectively, and the ratio of

blood urea nitrogen to nonprotein nitrogen was approximately normal, *i.e.*, 1:2. In the remaining 8 the urea nitrogen levels were subnormal, ranging from 4 to 12.3 mg. per cent and the blood urea nitrogen/non-

TABLE IX

Nonprotein Nitrogen and Urea Nitrogen in Representative Cases of Epidemic Hepatitis in Which the Duration of Disease Ranged from 3 to 12 Days

Case no.	Onset	Death	Date of examination	Nonprotein nitrogen (mg./100 cc. of blood)	Blood urea nitrogen (mg. per 100 cc. of blood)
104032	12/3/43	12/12	1/10	40	
107351	3/16/44	3/20	3/19	55	
			3/20	72	
114638	3/10/44	3/15	3/14	27	
114947	6/3/44	6/12	6/12	60	
119114	9/1/44	9/8	9/7	35	
119183	7/22/44	7/31	7/26	40	
122399	9/5/44	9/9	9/8	50	
			9/9	48	
122680	11/29/44	12/5	12/4	42	
124566	10/10/44	10/15	10/14	43	
125602	7/14/44	7/23	7/22	48	
126048	9/9/44	9/12	9/11	56	
126049	9/7/44	9/12	9/12	50.5	12.3
126108	9/14/44	9/22	9/19	44.4	
			9/21	45.6	
126811	1/9/45	1/13	1/12	36.7	
128003	1/18/45	1/29	1/25	31	16
128072	1/7/45	1/18	1/15	32	4
128558	10/28/44	11/9	11/7	52	
128577	10/28/44	11/5	11/5	60	
128713	11/7/44	11/14	11/11	43	
			11/13	39	5.9
128714	11/11/44	11/16	11/14	36.5	
			11/15	35	
			11/16	35	5.6
129240	1/23/45	2/1	1/25	33	6.2
133205	1/19/45	1/30	1/28	72	
133183	7/30/44	8/4	8/3	103	
131875	12/8/44	12/19	12/19	20	6.2
133190	9/27/44	10/1	9/30	75	
133196	4/11/44	4/20	4/18	40	
133199	1/6/45	1/18	1/16	35	16.6
133201	12/5/44	12/11	12/10	27	5.4
133204	12/27/44	1/5	12/30	40	
131874	9/10/44	9/14	9/12	30	
131667	10/24/44	10/31	10/27	42.8	8.2

protein nitrogen ratios correspondingly varied from 1:3.1 to 1:8. The subnormal levels, both absolute and relative, clearly indicate decreased urea formation. As this substance is formed solely in the liver,[38,39] a decrease in blood concentration is important evidence of hepatic inefficiency.

In the group studied no relation was apparent between duration of disease and nitrogen retention.

Blood Sugar. In destructive disease of the liver the blood sugar would be expected to fall to a low level.[39] In most cases of this series such a fall was obscured by the therapeutic administration of glucose. There were, however, 16 cases in which low values were obtained (Table X). In these cases the determinations were made at a time when the patients exhibited central nervous manifestations, *i.e.*, when they were in the terminal stage. The values ranged from 35 to 73 mg. per cent; in 9 they were below, in the remainder above, 55 mg. per cent.

Illustrative Cases

Pertinent clinical data and laboratory findings are given for 21 representative cases, in which the clinical duration of the disease ranged from 1 to 11 days.

Table X

Low Blood Sugar in Cases of Fulminant Hepatitis in Which the Duration of the Disease Ranged from 3 to 11 Days

Case no.	Onset	Death	Date of examination	Blood sugar (mg. per 100 cc. of blood)	Remarks (date of examination)
104274	5/16/44	5/23	5/22	38	Drowsy
114638	3/10/44	3/15	3/15	72	Comatose, convulsions
119114	9/1/44	9/8	9/7	37	Alternating restlessness and stupor
122680	11/29/44	12/5	12/4	40	Comatose
122399	9/5/44	9/9	9/9	40	Shock
119183	7/22/44	7/31	7/25	51	Stuporous
124971	10/22/44	10/29	10/26	62	Disoriented, delirious
125482	11/19/44	11/30	11/28	59	Irrational
126048	9/9/44	9/12	9/11	40	Semicomatose, shock
126049	9/7/44	9/12	9/12	35	Comatose, opisthotonos, shock
127334	12/11/44	12/15	12/14	73	Restless
128003	1/18/45	1/29	1/26	63	Semicomatose, convulsions
133205	1/19/45	1/30	1/28	56	Comatose
133182	7/22/44	7/31	7/25	51	Comatose
141771	9/18/44	9/21	9/20	66	Comatose
133190	9/27/44	10/1	10/1	50	Comatose, convulsions

In cases 1 to 10 the predominant manifestations at the onset were those common to a variety of acute infections. Some patients of this group presented serious diagnostic problems; thus, in cases 1, 7, and 10, malaria was suspected. In the second group of cases, 11 to 16, the clinical course of hepatitis was of the more familiar type, being ushered in by gastrointestinal symptoms. A third group (cases 17 to 19) represented the mixed infectious and gastrointestinal form. Case 20 is an example of recurrent hepatitis. In case 21 the first manifestation of the disease was mental disturbance.

In the examples given, 2 patients (cases 2 and 5) remained free from jaundice throughout the course of the disease; in one other (case 7) only the scleras became faintly icteric. In 3 patients (cases 4, 6, and 7) the icterus index did not exceed 35. Six patients (cases 2, 4, 5, 6, 11, and 16) passed into a state of shock. In 5 cases (nos. 2, 4, 6, 8, 10, and 12) the blood sugar sank to low levels. In one case (no. 6) cerebral manifestations led to exploratory craniotomy.

Approximately one-half of these patients had sustained combat trauma and presumably received transfusions of plasma or blood; one patient, not wounded (case 15), had received multiple transfusions of plasma for the treatment of hypoproteinemia. It will be noted that the clinical features of hepatitis were the same in patients who had previously been wounded and received transfusions as in those not wounded, *i.e.*, who had the "naturally" occurring form of hepatitis.

Case 1

The patient (Army Institute of Pathology, 124057) was a white soldier, 22 years of age.

Clinical Course. On April 2, 1944, he complained of generalized aching, occipital headache, fever, nausea, and several bouts of vomiting. His temperature was 102.4° F.; later in the day it declined to 99.8° F.; pulse was 76; respirations, 18. There was rigidity of the voluntary muscles in the right upper quadrant and tenderness over the liver. Icterus became evident on April 4; the patient was restless, hyperactive, had severe pain in the upper abdomen, was persistently nauseated, and vomited repeatedly. His temperature was 96.4° F.; pulse, 90; respirations, 20. On April 5, he became deeply comatose. His pupils were moderately enlarged; there was left external strabismus. Abundant coffee-ground material oozed from mouth and nostrils. The liver was soft and its edge palpable one fingerbreadth below the costal margin. The pulse was of good quality. An algid type of malaria with secondary hepatitis was suspected, but no parasites were found on repeated examination of the blood. During the day the patient's temperature rose to 104.4° F., pulse to 152, respirations to 32; blood pressure dropped from 144/80 to 94/74 mm. Hg. Death occurred on April 5; duration of disease, 3 days.

Laboratory Findings. (April 2.) *Blood:* red blood cells, 4.9 million; white blood cells, 4,700, with 51 per cent polymorphonuclear leukocytes and 49 per cent lymphocytes and monocytes. *Urine:* specific gravity, 1030; 1 plus albumin. (April 4.) *Urine:* 1 plus bile. *Icterus index:* 54.

(For illustrations of lesions see Figs. 9, 10, 12, and 19.)

Case 2

The patient (Army Institute of Pathology, 126048) was a white soldier, 34 years of age.

Clinical Course. On July 29, 1944, he was wounded in action by shell fragments, suffering compound comminuted fractures of the left radius, and penetrating wounds of the left thigh and eye. Details of treatment other than debridement were not recorded. He improved steadily and was convalescing at a normal rate. On September 9 the patient felt chilly, had a fever of 103° F., and was nauseated. Examination of the throat, chest, abdomen, and nervous system revealed nothing of note. He felt "stronger" on September 10, but complained of headache and

appeared to be dull and apathetic; temperature was 100.8° F. He vomited a dark brown fluid on three occasions. Physical examination was still negative. On September 11 a sudden change took place; the abdomen became rigid; rectal temperature fell to 94° F.; pulse was rapid; respirations were intermittent and averaged from 40 to 60 per minute. Chest examination was negative. During the day the patient became semicomatose. The blood pressure dropped to 80/40 mm. Hg. The neck was not rigid; the eyes were open, the pupils widely dilated; the left eye was fixed to light, the right reacted slowly. The patient was treated for shock and the next day seemed slightly improved, with a temperature of 99° F., a pulse of 120, and respirations 40 and regular. During the day he became restless. Respirations became shallow and averaged 52 per minute. The temperature rose to 102° F. Jaundice did not develop during the course of the illness; the scleras remained clear. Death occurred on September 12; duration of disease, 3 days.

Laboratory Findings. (September 11.) *Blood:* red blood cells, 4.5 million; white blood cells, 11,750, with 72 per cent polymorphonuclear leukocytes and 28 per cent lymphocytes; nonprotein nitrogen, 56 mg. per cent; blood sugar, 40 mg. per cent. *Spinal fluid:* protein, 30 mg. per cent; sugar, 28.5 mg. per cent; white blood cells, 9,000; globulin, negative; color, light straw yellow.

(For illustrations of lesions see Figs. 3, 17, 18, and 23.)

Case 3

The patient (Army Institute of Pathology, 133177) was a white soldier, 31 years, of age.

Clinical Course. On June 16, 1944, he complained of severe backache, headache, and intermittent chills. Temperature was 101.6° F.; pulse, 88; respirations, 20. On June 17 he experienced severe nausea with occasional vomiting. The liver was enlarged to 2 fingersbreadth below the costal margin, and was moderately tender. His scleras were icteric. On June 18 the temperature had dropped below normal; vomiting was persistent. That night the patient was extremely restless; he hiccoughed and vomited repeatedly. On June 19 he became stuporous, tossed about, and failed to respond to questions. Pupils were contracted; the remainder of the physical examination was negative; blood pressure was 110/80 mm. Hg. The patient sank into coma; pulmonary edema developed. Death occurred on June 20; duration of disease, 4 days.

Laboratory Findings. (June 17.) *Icterus index,* 65. *Urine:* 2 plus albumin; 2 plus bile. (June 19.) *Urine:* 1 plus albumin; 2 plus bile.

Case 4

The patient (Army Institute of Pathology, 122399) was a white soldier, 30 years of age.

Clinical Course. On June 26, 1944, he was wounded in action by a mortar shell, suffering multiple perforating wounds of bones and chest, and compound fractures of the right humerus and left tibia. Treatment consisted of debridement, plasma, sulfathiazine, and penicillin. On July 4 the left leg was amputated; healing of wounds progressed satisfactorily.

On September 5 he had a chill and a fever of 103.4° F.; he complained of headache and diarrhea. Jaundice developed on either September 5 or 6. The patient seemed improved during the day of September 7, but he sweated profusely that night, and on September 8 suddenly went into shock from which he failed to rally despite transfusions of plasma and other treatment. The skin was cold, the pulse thready, and the blood pressure not measurable. The temperature fell to 97.4° F.; later in the day, however, it rose to 102° and the blood pressure to 100/70 mm. Hg; respirations became shallow and rapid. Death occurred on September 9; duration of disease, 4 days.

Chemical Examination of the Blood

Date	Icterus index	Nonprotein nitrogen (*mg. per cent*)	Sugar (*mg. per cent*)
Sept. 8	20	50	133
Sept. 9	33	48	40

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes and monocytes (*per cent*)
Sept. 6	4.2	5,000	52	48
Sept. 7		6,500		
Sept. 8	4.7	13,000	54	45

Urine

Sept. 6 Albumin, negative
Sept. 8 Albumin, trace
Sept. 9 Albumin, + +

Agglutinations

Sept. 7 Heterophil, negative; typhoid "O," negative
Typhoid "H," negative; *Leptospira icterohaemorrhagiae:* negative

Case 5

The patient (Army Institute of Pathology, 126423) was a white soldier, 25 years of age.

Clinical Course. He was wounded in action by a rifle bullet on July 25, 1944. Laparotomy the following day revealed a laceration of the posterior wall of the bladder, multiple lacerations of the ileum, a laceration of the sigmoid, and the peritoneal cavity full of blood and urine. Transfusions, plasma, penicillin, and sulfadiazine were given and by August 5 he was strong enough to be transferred to a general hospital. On arrival he was found to have a functioning colostomy, a suprapubic cystotomy, a urinary fistula through the right buttock, and a pleural effusion. The effusion cleared spontaneously, and despite an episode of thrombophlebitis in the left leg it was possible to deal surgically with his fistulas. Improvement was gradual but steady, and he was evacuated to the United States on October 13. On October 14 his temperature suddenly rose to 104° F. Physical examination and roentgenologic examination of the chest and abdomen were negative. The white blood cell count was 10,150. At 9:30 a.m. on October 15 he vomited; by 1:00 p.m. he was restless, with signs of impending shock. He began to vomit coffee-ground material and passed tarry stools. His white count rose to 19,000, with 72 per cent polymorphonuclear leukocytes and 28 per cent lymphocytes. At 4:15 a.m. on October 16 he began to cough up blood-tinged sputum. Blood pressure was 120/84 mm. Hg; pulse, 116; respirations, 14. At 6:25 a.m. he had a convulsive seizure and went into profound shock. Jaundice did not develop during the course of the illness. Death occurred on October 17; duration of disease, 4 days.

(The cut surface of the liver is shown in Fig. 4.)

Case 6

The patient (Army Institute of Pathology, 126049) was a white soldier, 19 years of age.

Clinical Course. On July 18, 1944, he was wounded in action, receiving multiple

bullet wounds of the right upper arm which produced a severe compound comminuted fracture of the humerus. Secondary closure of the wounds was done and he convalesced rapidly. On September 7 generalized aching developed and his temperature rose to 102.8° F. On September 8 he complained of nausea; on September 9 his temperature was 101° F. General physical examination was negative, and when the cast was removed in order to inspect the wounds, they were found to be healing satisfactorily. On September 11, although the temperature was normal, nausea and vomiting persisted. The patient fell out of bed during the night and afterward seemed disoriented, although physical examination was still negative. The following day, September 12, jaundice was evident. Irrationality was rapidly followed by deep coma, interrupted by repeated convulsive seizures, some of which were associated with opisthotonos. The pupils were equal; no papilledema was present. There were bilateral positive Babinski signs, and bilateral sustained ankle clonus; abdominal and cremasteric reflexes were absent, as were Chvostek's and Trousseau's signs. It was thought that a subdural hematoma might have resulted from the fall out of bed; however, cranial exploration showed no evidence of intracranial bleeding and the ventriculogram was normal. Blood pressure fell during the operation from 190/100 to 70/55 mm. Hg. Death occurred on September 12; duration of disease, 5 days.

Laboratory Findings. (September 11.) *Blood:* white blood cells, 8,250. (September 12.) Chemical examination: nonprotein nitrogen, 50.5 mg. per cent; urea nitrogen, 12.3 mg. per cent; sugar, 35 mg. per cent; icterus index, 35. *Spinal fluid:* total protein, 20 mg. per cent; cells, 22 (16 polymorphonuclear leukocytes and 6 lymphocytes and monocytes). *Urine:* color, dark amber; specific gravity, 1.027; albumin, 3 plus; sugar, negative; acetone, 1 plus; diacetic acid, negative.

Case 7

The patient (Army Institute of Pathology, 133183) was a white soldier, 26 years of age.

Clinical Course. On July 30, 1944, he complained of chills, fever, headache, and general aching which came on suddenly and persisted for several days, accompanied by anorexia, nausea and vomiting. He was admitted to the hospital on August 2 when he appeared acutely ill. Temperature was 102.6° F.; pulse, 100; respirations, 20. The only positive finding on physical examination was a palpable and tender spleen. A tentative diagnosis of tertian malarial fever was made; however, repeated blood smears were negative for malarial parasites. There was no improvement during the night and on the morning of August 3 the temperature had risen to 104.4° F.; pulse, 96; respirations, 20. The patient complained of headache, and pain in the chest. The icterus index was 28; the blood nonprotein nitrogen, 103 mg. per cent. The physical findings were unchanged. During the day the temperature subsided to 98° F. and by the next morning, August 4, to 96.4°. He became disoriented and had hallucinations. The liver and spleen were palpable. The lungs appeared clear; there was no roentgenologic evidence of pneumonic consolidation or fluid. The heart was normal. The deep reflexes were hyperactive; the superficial reflexes diminished. During the day the patient gradually became cyanotic. Definite jaundice never developed and only a faint icteric tint was noted in the scleras post-mortem. Death occurred the evening of August 4; duration of disease, 6 days.

Case 8

The patient (Army Institute of Pathology, 124971) was a white soldier, 24 years of age.

Clinical Course. He was wounded in action by an exploding shell on August 13, 1944, sustaining damage to both orbital regions. For several weeks he continued to have a draining orbital sinus; his eyesight was lost; his general physical

condition was good. On October 22 he awakened with a severe throbbing headache and during the day his temperature was between 99° and 100° F. On October 23, it rose to 103.5° F.; he vomited and complained of considerable muscular aching. His skin took on an icteric tint; no other physical findings of significance were noted. Between October 23 and 26 jaundice deepened, and his condition became progressively worse. The temperature remained between 99° and 100° F. He was disoriented. On October 29 he had several generalized convulsions and became deeply comatose. Death occurred on October 29; duration of disease, 7 days.

Chemical Examination of the Blood

Date	Icterus index	Sugar (*mg. per cent*)	Urea nitrogen (*mg. per cent*)	Serum protein (*gm. per cent*)	Albumin (*gm. per cent*)	Globulin (*gm. per cent*)
Oct. 25	33					
Oct. 26	64	62	9.3	5.2	3.3	1.9

Blood Counts

Date	Red blood cells (*millions*)	White blood cells
Oct. 22	4.0	5,900
Oct. 23		6,500

Case 9

The patient (Army Institute of Pathology, 125602) was a white soldier, 30 years of age.

Clinical Course. On July 14, 1944, malaise and general aching began and the patient was admitted to the hospital. His temperature was 104° F.; he was not jaundiced; the only significant physical finding was enlargement of the liver. On July 18 a moderate degree of jaundice of the skin and scleras was noted. On July 21 the patient became drowsy and difficult to arouse, later sinking into coma. There was a terminal fever of 106° F. Death occurred on July 21; duration of disease, 7 days.

Chemical Examination of the Blood

Date	Icterus index	Nonprotein nitrogen (*mg. per cent*)	Blood sugar (*mg. per cent*)
July 19	54		
July 22	108	48	100

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes and monocytes (*per cent*)
July 19	5.12	5050		
July 21		10400	57	43

Urine

Date	Albumin	Bile
July 19	++	
July 21	++	+

(For photomicrograph of kidney see Fig. 30.)

Case 10

The patient (Army Institute of Pathology, 133205) was a white male, 25 years of age.

Clinical Course. On January 19 his illness was ushered in with ocular headache, general aching, chills, fever, loss of appetite, and mild nausea. He was admitted to the dispensary on January 20. Symptoms became progressively worse during the next 3 days, and on January 23 jaundice became evident. The patient was sent to the hospital with a diagnosis of malaria or hepatitis. On admission, temperature was 100.8° F.; pulse, 82; respirations, 16. He complained of headaches, mild abdominal pain, complete loss of appetite, occasional nausea and vomiting. Physical examination revealed moderate jaundice; the liver was slightly enlarged and tender; the spleen also was enlarged and tender; the chest was clear. Neurologic examination was negative. On January 25 the patient appeared slightly improved; he was afebrile, but liver and spleen were very tender, and icterus was deepening. He vomited during the night. On January 27 he was very drowsy but could be aroused easily, was well oriented, looked critically ill, and had vomited two or three times. By January 28 the patient was deeply jaundiced; he was in coma but responded to painful stimuli. The liver was palpable two fingersbreadth below the costal margin. On January 29 the coma deepened; there was some rigidity of the extremities; pulmonary edema developed. Death occurred on January 30; duration of disease, 11 days.

Chemical Examination of the Blood

Date	Nonprotein nitrogen (*mg. per cent*)	Blood sugar (*mg. per cent*)	Icterus index
Jan. 27			133
Jan. 28	72	56	124

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes and monocytes (*per cent*)
Jan. 24	4.9	9,000	74	26
Jan. 27	4.7	8,000	71	29
Jan. 29	4.5	10,000	76	24

Blood Examinations for Malarial Parasites

Jan. 24, 25, 26, 27, 28, and 29 Negative

Case 11

The patient (Army Institute of Pathology, 126811) was a white soldier, 31 years of age.

Clinical Course. On October 3 he received a perforating gunshot wound of the abdomen necessitating laparotomy and colostomy. He made a good recovery, gained 10 pounds, and felt well for the next 3 months. On December 28 the colostomy was closed under local anesthesia; the postoperative course was uneventful. On January 9 he complained of pain in the epigastrium, became nauseated and vomited. On January 11 he was still slightly nauseated; the abdomen was soft and flat; the lungs were clear. On January 12 temperature mounted to 104° F.; the patient vomited and was nauseated; by evening he was somewhat disoriented. The conjunctiva was definitely icteric; the urine was dark; the stools light brown; the abdomen was soft and tender over both the hepatic and splenic regions, but the liver was not palpable. On January 13 the patient suddenly collapsed; the

pulse became very rapid and almost imperceptible; respiration was strained as in marked air hunger; blood pressure fell to 84/66 mm. Hg; temperature was 103.2° F. by rectum. The patient was alternately comatose and awake and restless. Blood pressure measurements were made repeatedly and, although they could not be accurately obtained, approximated 88/60 mm. Hg. The pulse became very thready and weak, the breathing shallow. Death occurred on January 13; duration of disease, 4 days.

Laboratory Findings. Chemical examination of the blood: Nonprotein nitrogen (January 12), 37 mg. per cent; icterus index (January 13), 60. *Urine* (January 12): albumin, 2 plus; bile, 0.

(For photomicrographs of kidney see Figs. 31 and 32.)

Case 12

The patient (Army Institute of Pathology, 128714) was a white soldier, 28 years of age.

Clinical Course. On January 9, 1944, he suffered severe burns on the legs, when gasoline spilled on his uniform and was ignited. The resulting burns were debrided, sulfadiazine ointment was applied, and he received plasma intravenously. On October 25 multiple grafts were applied to the wound and by November 5 they had taken. On November 11 the patient complained of abdominal fullness and distress. On November 13 he vomited, and also noticed that his skin had become yellow and his urine dark. On November 14 he vomited a great deal but had no other complaints. On November 15 his condition appeared good; blood pressure was 120/80 mm. Hg. He took small amounts of fluid eagerly and retained them. In the afternoon, however, he became increasingly restless and later delirious. Respirations were labored and pulse weak. Edema of the lungs was noted. Death occurred on November 16; duration of disease, 5 days.

Chemical Examination of the Blood

Date	Nonprotein nitrogen (*mg. per cent*)	Urea nitrogen (*mg. per cent*)	Icterus index	Blood sugar (*mg. per cent*)
Nov. 14	36		77	
Nov. 15	35			111
Nov. 16 (a.m.)	35	5.6		97
Nov. 16 (p.m.)	44	5.5	175	44

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes and monocytes (*per cent*)
Nov. 14	5.3	8,350	72	28
Nov. 16	5.2	16,450	90	10

Case 13

The patient (Army Institute of Pathology, 114947) was a Javanese soldier of the Dutch Army, 31 years of age.

Clinical Course. On or about June 3 he noticed vague abdominal pain and began to vomit. His stools became light in color. When he was admitted to the hospital on June 9 the temperature was normal, liver and spleen were neither palpable nor tender, and the abdomen was soft. The scleras were then normal,

but on June 10 became slightly icteric. The stools were clay-colored. The patient vomited coffee-ground material. On June 11 he became irrational, over-active, and then listless. On June 12 scleras were definitely icteric; the patient sank into coma. For the first time since the onset of symptoms there was fever, which rose to 104° F. Death occurred on June 12; duration of disease, 9 days.

Chemical Examination of the Blood

Date	Icterus index	Nonprotein nitrogen (*mg. per cent*)
June 11	85	
June 12	160	60

(For photomicrograph of liver see Fig. 11.)

Case 14

The patient (Army Institute of Pathology, 104032) was a white soldier, 22 years of age.

Clinical Course. On December 3, 1943, he complained of nausea and vomiting. He was admitted to the hospital on December 4, appearing neither acutely ill nor in discomfort; nausea and vomiting were not severe; there was slight tenderness in the upper abdomen. Temperature was 99° F.; pulse, 88; respirations, 22; blood pressure, 100/80 mm. Hg. On December 7 an icteric tint to the scleras was noticeable. During the next 2 days jaundice deepened rapidly and became intense by December 10. The liver edge was palpable. The patient became irrational, later comatose. Death occurred on January 12; duration of disease, 9 days.

Chemical Examination of the Blood

Date	Icterus index	Nonprotein nitrogen (*mg. per cent*)
Dec. 8	25	
Dec. 10		40
Dec. 11	75	

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes and monocytes (*per cent*)
Dec. 4	4.3	6,350	76	24
Dec. 5	4.3	6,000	60	40
Dec. 8	4.1	6,850	77	23
Dec. 10	4.8	5,100	79	21

Urine

Date	Bile
Dec. 4	0
Dec. 8	+
Dec. 10	+

Case 15

The patient (Army Institute of Pathology, 111844) was a white soldier, 22 years of age.

Clinical Course. He was admitted to the hospital in January, 1944, with edema of the hands and feet. Hypoproteinemia was found, without alteration of the albumin-globulin ratio. The origin of the protein deficiency was obscure; all tests for liver function were normal. He was placed on a suitable diet and given five plasma transfusions. Within 4 weeks his blood proteins had reached normal levels and he was discharged. On April 5 he complained of abdominal pain and nausea, and jaundice appeared. When the patient was admitted to the hospital on April 12 the liver was enlarged and tender and the urine dark brown. His condition deteriorated rapidly. On April 14 he was delirious, his abdomen was distended, and jaundice was deepening; coma ensued. The clinical course in the hospital was entirely afebrile. Death occurred on April 15; duration of disease, 10 days.

Chemical Examination of the Blood

Date	Icterus index	Urea nitrogen (*mg. per cent*)	Serum protein (*gm. per cent*)	Albumin (*gm. per cent*)	Globulin (*gm. per cent*)
April 12	60				
April 14	105	12	6.9	3.0	3.9
April 15	105	14	7.0	3.2	3.8

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes and monocytes (*per cent*)
April 12		7,500	63	37
April 14		9,150	70	30

(See Fig. 26 for photomicrograph of proliferating bile ducts.)

Case 16

The patient (Army Institute of Pathology, 128072) was a white soldier, 26 years of age.

Clinical Course. On October 23, 1944, he was wounded in action by an enemy mine, sustaining traumatic amputation of the lower part of the left leg. Early treatment included administration of sulfadiazine, penicillin, and transfusions of whole blood and blood plasma. Convalescence was uneventful; the soldier was returned to the United States and under further treatment the stump healed satisfactorily. On January 7, 1945, he complained of vague abdominal discomfort, loss of appetite, aversion to greasy foods, general malaise, nausea and vomiting. During the next few days, loss of appetite and abdominal discomfort continued, but nausea and vomiting subsided. On January 13 he had a mild attack of urticaria and became slightly jaundiced. The liver was not definitely palpable, but was slightly tender and increased in size to percussion. Diagnosis of acute hepatitis was made and treatment instituted. The patient's condition remained essentially unchanged until the morning of January 17, when he suddenly became confused, then disoriented, and finally stuporous and comatose. Oozing of bloody fluid from the mouth became profuse. Icterus deepened in intensity; the liver, which 3 days before had been somewhat enlarged, now was found to be definitely smaller to percussion. The blood pressure fell from 155/90 to 84/40 mm. Hg. There was a terminal rise of temperature to 105° F. Death occurred on January 18; duration of disease, 11 days.

Laboratory Findings. (January 15.) *Blood:* icterus index, 76; white blood cells, 10,400; (January 17) blood nonprotein nitrogen, 32 mg. per cent; blood urea nitrogen, 4 mg. per cent.

Case 17

The patient (Army Institute of Pathology, 129051) was a white soldier, 27 years of age.

Clinical Course. On August 7, 1944, he was wounded in action by a rifle bullet, sustaining a compound fracture of the left os calcis. A suppurating sinus persisted, and he was evacuated to the United States. Between November 15 and December 22 he was treated with penicillin. At Christmas he was well enough to receive a convalescent furlough. On his return, penicillin therapy was again instituted. Although evidence of healing of the bone defect could be seen in the roentgenogram, a sinus still persisted which required surgical treatment. Before the contemplated operation the patient was granted another furlough. On February 14, upon arriving at the home of his sister, he stated that he was not feeling well and complained of pain in the right hip, nausea and vomiting. It was noted that his scleras were yellowish and his skin slightly jaundiced. He declined to return to the hospital as he did not believe himself seriously ill. On February 15 he stayed overnight with a friend and on the morning of February 16 he was restless and at times irrational. He was taken by ambulance to an Army hospital, arriving on the morning of February 18. On admission he was semiconscious. His pupils were unequal, the left being larger than the right; both reacted to light. His neck was definitely rigid. Spinal puncture was done; the fluid was clear and apparently not under increased pressure. Physical examination disclosed slight icterus and hyperactive reflexes. The abdomen was soft and the lower extremities were spastic. Râles were heard over the chest. The diagnosis of meningitis was entertained although examination of the spinal fluid was not confirmatory. Pulmonary edema developed. Death occurred on February 18; duration of disease, 4 days.

(For photomicrograph of liver see Fig. 16.)

Case 18

The patient (Army Institute of Pathology, 128329) was a white soldier, 23 years of age.

Clinical Course. On May 26 anorexia, nausea, and fever developed, and he was admitted to the hospital on May 27. He appeared acutely ill; temperature was 100°, later 103° F. The spleen and liver were not palpable or tender. Chest examination was negative. On May 28 his general condition was improved; temperature dropped to 99.2° F., and subsequently remained normal. The patient was eating well. On May 31 jaundice was noted for the first time; he also complained of nausea. On June 1 he appeared ill, with lassitude, nausea and vomiting; jaundice deepened rapidly. On June 2 he became semistuporous and restless; there was slight tenderness in the upper abdomen. On June 3 profound coma set in, but there were no abnormal neurologic findings. Pulmonary edema developed. Death occurred on June 4; duration of disease, 9 days.

Chemical Examination of the Blood

Date	Icterus index	Urea nitrogen (*mg. per cent*)	Serum protein (*mg. per cent*)	Albumin (*gm. per cent*)	Globulin (*gm. per cent*)
May 31	65	5.7	5.8	3.5	2.3

Urine

Date	Albumin	Bile
May 28	++	o
May 31		+
June 2		+
June 4	++	

Case 19

The patient (Army Institute of Pathology, 129240) was a white soldier, 27 years of age.

Clinical Course. On October 23, 1944, he was wounded in action, suffering traumatic amputation of the left foot. He was treated by debridement, transfusions of plasma and whole blood, and penicillin. Convalescence was uneventful until January 23, 1945, when anorexia and nausea appeared and he noticed that his urine was becoming darker and his feces lighter than normal. Sensations of chilliness and general malaise were followed by a rise in temperature to 102° F. On January 24 he had a second chill; his temperature now rose to 104° F. He was jaundiced. The liver edge extended 2 fingersbreadth below the costal margin and was not tender. The spleen was easily palpable. On January 26 temperature fell to normal and remained between 97° and 99° F. for the next few days; nausea diminished; his appetite, however, remained very poor. On January 28 the patient suddenly became much worse; he vomited repeatedly; jaundice increased in intensity; the liver appeared to diminish in size; he became mentally confused and delirious; bled from the mouth and nose; and lapsed into coma. Death occurred on February 1; duration of disease, 9 days.

Chemical Examination of the Blood

Date	Icterus index	Nonprotein nitrogen (*mg. per cent*)	Serum protein (*gm. per cent*)
Jan. 25	33	36	6.2
Jan. 28	120		

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes and monocytes (*per cent*)
Jan. 25	4.5	5560	60	40

Repeated examinations of blood for malarial parasites were negative.

Case 20

The patient (Army Institute of Pathology, 133194) was a white soldier, 21 years of age.

Clinical Course. He had been hospitalized for "hepatitis without jaundice," once for 2 months, beginning April, 1944, and a second time for 4 months, beginning June 20, 1944. His symptoms consisted chiefly of distress in the upper abdomen radiating to the back, anorexia, and intolerance for greasy foods. He was also thought to be psychoneurotic with seclusiveness and dependency; a diagnosis of "anxiety state" had been made. On the third entry to the hospital, on November 6, physical examination was negative; liver and spleen were not palpable. Laboratory studies the week after admission were negative; icterus index, 8; cephalin flocculation, 0/0; blood phosphatase, 4.4 units. Hemoglobin, 85 per cent; red blood cells, 4,500,000; white cells, 5,250, with 62 per cent polymorphonuclear leukocytes and 38 per cent lymphocytes. He complained of constant upper abdominal pain throughout the several weeks he was observed as a psychoneurotic patient. On November 22 he complained of headache, anorexia, and nausea. On November 23 he felt feverish; temperature rose to 100° F.; abdominal pain became more severe. Physical examination showed tenderness but no rigidity in the right upper quadrant. That evening he had a chill lasting 30 minutes. The morning urine,

November 24, was dark; the scleras were questionably icteric. Tenderness in the right upper quadrant persisted, but the liver could not be felt. By November 28 the scleras were definitely icteric and the icterus index was 27. He became acutely ill with severe vomiting. The right upper quadrant was exquisitely tender. On November 30 restlessness alternated with lethargy, vomiting continued, and the patient went into coma. Death occurred on December 1; duration of disease, uncertain (terminal illness, 8 days).

Case 21

The patient (Army Institute of Pathology, 123465) was a white soldier, 25 years of age.

Clinical Course. On July 30, 1944, he was admitted to a clearing company in a state of mental agitation. The diagnosis was "battle fatigue." While on the ward he became disturbed, fearing Japs were going to attack him. He had no other complaints and physical examination was negative except for evidence of recent loss of weight. There was no jaundice. Under mild sedation he became somewhat quieter. In the evening of the same day his respiration ceased suddenly and no pulse was perceptible. Death was thought to have occurred instantly, due either to cardiac stoppage as a result of vagus stimulation secondary to fright, or to pulmonary embolism. Death occurred on July 30; duration of disease, uncertain (clinically, 1 day).

(For photomicrographs of liver see Figs. 8, 15, 24, and 27.)

Pathologic Anatomy

In fulminant hepatitis, lesions other than those in the liver are not striking. The changes in the gallbladder, extrahepatic bile ducts, regional lymph nodes, bone marrow, and the hemorrhagic phenomena are of the same general type as described previously. We may limit ourselves, therefore, to accounts of the liver, ascites, spleen, intestines, kidney, and brain.* Consideration of the prostate will be reserved for a future communication. No noteworthy pathologic changes were found in the other organs.

Similarity of Lesions of "Spontaneous" and "Inoculation" Hepatitis. The lesions of the several epidemiologic forms of hepatitis included in this study are in every respect indistinguishable. Hence an account of the pathologic anatomy may be given for the entire series.

LIVER

Two processes characterize the pathologic picture of the liver in fulminant hepatitis: extreme and often complete destruction of liver cells, and marked inflammatory response. From naked-eye examination of the organ, however, it is not possible to surmise the extensive destruction of the parenchyma or the degree of inflammation. The vague term "acute yellow atrophy," although connoting rapid break-

* We are grateful to Lt. Col. Philip Custer for examining many sections of spleen, and to Dr. Nathan Malamud and Major Webb Haymaker for advice regarding the changes in the brain.

down of hepatic parenchyma, is not descriptive of the appearance, and cannot properly be applied.

Gross Appearance

In the great majority of cases the liver is reduced in size, but the shrinkage is usually moderate. The weights are given in Table XI: in one-half they range between 1000 and 1400 gm., and in approximately one-third of the series they are below 1000 gm. Excessive reduction to below 800 gm. occurs in only 6 per cent, and, at the other extreme, weights exceed 1600 gm. in 11 per cent. The median for the entire group is 1150 gm. The data upon which Table XI is based are graphically analyzed in Text-Figure 7 (p. 904), first, with respect

TABLE XI

Weight of Liver in 101 Cases of Fulminant Hepatitis

	No. of cases	Associated with ascites
Below 800 grams	6	1
800 —999	25	5
1000—1199	28	9
1200—1399	22	6
1400—1599	9	1
1600—1799	8	6
Over 1800	3	2
Total	101	30

to duration of disease; second, with reference to the two main epidemiologic forms (represented in the figure as "wounded" and "not wounded"), and, third, in relation to the presence or absence of ascites. Great scattering of weight and absence of correlation with duration are evident. For example, the livers from the 12 cases having a duration of 4 days weigh from 600 to 1800 gm., with a median of 1200 gm. Livers from cases having durations of only 2 or 3 days present as great shrinkage or as little change in size as those with a longer course. Livers from fatal cases of "naturally" occurring hepatitis do not differ significantly in weight from those of "inoculation" hepatitis. The relation to ascites will be discussed later.

The surface is always either smooth or finely wrinkled (Fig. 1); it is never deformed by the nodular or tumor-like elevations characteristic of the subacute stage. The shape is little altered, except that the anterior edge commonly is sharp. The capsule is usually transparent. Small subcapsular hemorrhages are common. The color is not distinctive and varies through shades of red, purple, and brown; mottling is common and such combinations as gray-red and purple, or tan and red, are often observed. The organ is usually soft and flabby, sometimes to

such an extent that it flattens when laid on the table; but the flabbiness may be masked by such great engorgement that the organ is tense.

The cut surface nearly always shows diffuse involvement, most frequently in the form of an exaggerated "nutmeg" pattern (Figs. 3 to 7). The lobular peripheries are outlined by pale grayish or yellow-gray bands of variable width, which contrast with the dark red or purple of the slightly sunken inner part of the lobules. In other cases, on the contrary, the landmarks are so indistinct that the cut surface resembles that of an acutely congested spleen (Fig. 2). The blood content also varies considerably; in some instances the inner zones of every lobule ooze blood copiously, in others the entire organ is relatively ischemic; sometimes areas of engorgement and relative bloodlessness alternate. The cut surfaces nowhere have a greasy sheen; the bands which outline the lobules have the fresh appearance of healthy tissue; the lobular interiors, when not too greatly congested, are dull grayish red, but not fatty.

In roughly 10 per cent of the cases the changes are most marked in the left lobe, which is more shrunken and has a more wrinkled surface.

Microscopic Appearance

In this section we shall deal principally with the lesions typical of the fulminant form of hepatitis in which the clinical duration of disease is less than 10 days. Later a brief account will be given of the changes found in two other groups of cases, namely, those which survive from 10 to 19 days, and those of unknown clinical duration. These two groups are important because they not only show transition stages to the subacute form previously studied, but they also illustrate the disparity which may exist between the clinical course and the anatomic changes.

As already stated, two processes dominate the microscopic picture of fulminant hepatitis: extensive destruction of liver cells and marked inflammatory reactions. Regenerative hyperplasia of surviving parenchyma, which is so prominent a feature of the subacute form, is lacking or minimal, and is confined to biliary rather than to hepatic epithelium.

Destruction of Liver Cells. The destructive process, in the majority of cases, involves all parts of the liver uniformly. As a rule, destruction of liver cells is extensive and, in many instances, complete (Figs. 8, 9, and 15); but often a narrow rim, a few cells in width, persists at the lobular periphery (Fig. 16). Very rarely the destruction instead of being predominantly central is peripheral, sparing small isolated patches of parenchyma in the interior.

In a few of the most rapidly fatal cases a scattering of shadow forms

of liver cells sometimes remain but it is distinctive that the dead cells are removed rapidly. In the entire series no example of the earliest stage in the necrotic process was encountered; cell destruction was always advanced. As a rule, even in cases with a clinical history of only 3 or 4 days no traces of dead cells were found. These facts suggest that the agent bringing about necrosis effects speedy lysis and enzymic digestion rather than cell coagulation as seen in the necrosis of anoxemia and after many hepatic poisons. Less commonly the few remaining cells show degenerative changes, such as small irregular droplets of fat or other vacuoles in the cytoplasm. These changes are regarded as secondary to the profound disturbances of blood supply that accompany necrosis of the parenchyma.

Persistence of Reticulum and of Sinusoids. The destructive process specifically affects liver cells; the other components of the lobule escape. The reticulum, in all cases, is intact (Fig. 17). The inter-reticular spaces, formerly occupied by liver cells, in some instances become packed with monocytes and erythrocytes and little shrinkage occurs (Fig. 21); in other instances the meshes are empty and collapsed. The sinusoids usually are widely engorged (Figs. 9 and 22); small hemorrhages occasionally occur. In brief, the liver in fulminant hepatitis is rapidly reduced to a spongy framework, infiltrated by inflammatory cells and often distended with blood.

Inflammatory Reaction. In the present series, inflammatory cellular infiltration is considerably more marked than in the subacute form. The cell reaction is most conspicuous at the lobular periphery, *i.e.*, in the portal stroma and the interlobular boundary (the "septum vasculare").[10,19,20] Within the lobular remnants inflammation is less prominent and of somewhat different type.

Often whole lobules are outlined by bands of densely packed cells (Figs. 8 and 9). The composition of the infiltrate varies from case to case; a fairly representative picture is shown in Figure 11. It will be noted that the cells are well preserved and that there is no indication of breakdown. They are chiefly mononuclear forms—reticulo-endothelial derivatives, plasma cells, and lymphocytes—but neutrophils and eosinophils in relatively small numbers are almost invariably present (Fig 13).

Within the lobular remnants, mobilized and proliferated macrophages predominate; frequently they become so large and numerous as to give a spurious appearance of a "parenchyma" consisting of disunited but preserved liver cells (Fig. 14). The macrophages commonly contain a brownish, sudanophilic, and faintly acid-fast pigment, lipofuscin, which probably is derived from the disintegrated hepatic cells.

Lymphocytes, plasma cells, and granulocytes, while not numerous, are present also.

Inclusion bodies, cytoplasmic or intranuclear, such as occur in many different virus infections, are not encountered in any kind of cell.

Efferent Veins. In the subacute form of hepatitis of the 1942 series, endophlebitis of the efferent veins was a conspicuous, though not a pathognomonic, feature. In the fulminant form it is less commonly encountered. Frequently, however, the walls of the central lobular and smaller collecting veins are much thickened and of homogenous, hyaline texture (Fig. 18).

Regeneration of Liver Cells. In no case of the fulminant group is there any noteworthy degree of regenerative hyperplasia. At most, the persistent cells are large, irregular in outline, and multinucleated; significant increase in number is not apparent. Regeneration obviously cannot take place when destruction of the parenchyma is complete.

Proliferative Changes in Bile Ducts. It is of importance to note that in most cases, even the most acute, the small twigs of the bile ducts, both septal (perilobular) and interlobular, exhibit some evidence of proliferation. These little ducts normally are inconspicuous. The degree of proliferation attained is illustrated by representative photomicrographs. In Figure 23 is shown a septal duct composed of large, closely packed cells with prominent nuclei; the duration of disease in this case was only 3 days. In Figure 24 may be seen a more conspicuously proliferating duct lying in the portal stroma; the duration in this case was, clinically, less than 1 day. In 2 cases with duration of 9 or 10 days, the ducts have irregular shapes, due to budding and branching, and the nuclei of the component cells are deeply chromatic (Figs. 25 and 26).

These examples illustrate the rapid proliferation of which biliary epithelium is capable.

Lesion in Patients Surviving from 10 to 19 Days. In approximately one-third of the patients surviving 10 to 19 days the lesions were identical with those of the more fulminant cases. Thus, in some instances, the microscopic picture of the liver from patients who survived for 10 or more days was indistinguishable from that when death occurred within 3 or 4 days after onset. More often, however, the lesions were similar to those encountered at corresponding periods of survival in the 1942 series. In agreement with the findings in that series the involvement of the liver in the less fulminant group is not uniform. This difference is shown in Figures 28 and 29, which represent fields from two hepatic areas of a patient who survived for 12 days. Figure 28 shows a region in which destruction of hepatic parenchyma is in-

complete, while Figure 29 is from an area in which destruction is practically complete and the lobular remnants are outlined by proliferating bile ducts.

Correlation between Gross and Microscopic Changes. The degree of engorgement and of inflammatory infiltration are clearly correlated with size and appearance of the liver. As to the former, the content of blood accounts for the great variations of weight, despite the fact that in all cases only traces of parenchyma remain. The predominant "nutmeg" pattern of the cut surface is brought about by a combination of the marked inflammatory infiltration at the lobular periphery and the engorgement in the interior of the lobular remnants. In cases where the cut surface resembles that of a congested spleen, engorgement outbalances inflammation.

As has been stated, the gross appearance of the liver gives no indication of the extreme destruction of the parenchyma.

Disparity between Lesions and Clinical Duration

The clinical duration is not always correlative with the pathologic findings; sometimes the process is obviously older than the history suggests, less often the reverse is true. For example, in many instances no relation can be established between duration of disease and degree of inflammatory reaction; its severity in some of the shortest cases makes the conclusion inescapable that the reaction must have antedated the first symptoms. Similarly, there is often no correspondence to the extent of parenchymatous destruction or to the degree of proliferation of the small bile ducts. For example, the lesions shown in Figures 8, 15, 24, and 27 are from a patient with a clinical course of less than 1 day; but all traces of dead liver cells have been removed and the bile ducts exhibit active proliferation. In numerous other very early cases like pictures are obtained. Again, in some of the most fulminant cases, jaundice appeared on the first day; no doubt disintegration of the liver was advanced before symptoms began.

An even greater discrepancy between clinical history and pathologic changes was noted in a group of 18 cases which purposely were omitted from the analysis of duration given in Tables I, II, IV, and XII. In this group, 5 cases with a short course showed evidence of well marked regeneration with nodularity of the livers. In contrast, 8 cases prolonged for more than 20 days presented the acute changes characteristic of the fulminant form. It is possible that some of the latter were recurrent infections which ran an acute course.

It may be concluded that in the more rapidly fatal cases the lesions are older than the clinical manifestations; in other words, when symp-

toms appear the liver is already definitely involved; in some instances the clinical course may be "silent," or the symptoms minimal even when death occurs within a few days. These conclusions are borne out by the studies of specimens taken for biopsy at different stages of the disease in nonfatal cases; here also the lesions frequently appear older than the clinical duration.[19,40]

Ascites

The incidence of ascites has been tabulated for 140 cases (Table XII). There is a definite correlation between ascites and clinical duration of disease: the incidence rose from 24 per cent when death occurred within 10 days, to 43 per cent when it was delayed from 10

TABLE XII
Occurrence of Ascites in 140 Cases of Hepatitis
(New Series)

Duration of disease	Total no. of cases	Ascites	Per cent of cases
To 9 days	68	16	24
10—19 days	33	14	43
Over 19 days	39	27	70

to 19 days, and to 70 per cent when the course was more protracted. The amount of fluid in the fulminant cases was usually less than 1 liter, whereas in the others, larger volumes (2 or 3 liters) were the rule.

The relation between ascites, weight of liver, and duration of disease is shown for wounded and nonwounded cases in a distribution graph (Text-Fig. 7). It will be noted that ascites occurred even in the most fulminant cases. The relation to weight of liver is interesting: ascites was more common when the liver was but little shrunken or was actually enlarged, than when its bulk was considerably decreased. Thus, in the 25 cases with livers weighing above 1300 gm. ascites was observed 11 times, whereas it occurred only 4 times in the 25 cases having the smallest livers (below 920 gm.).

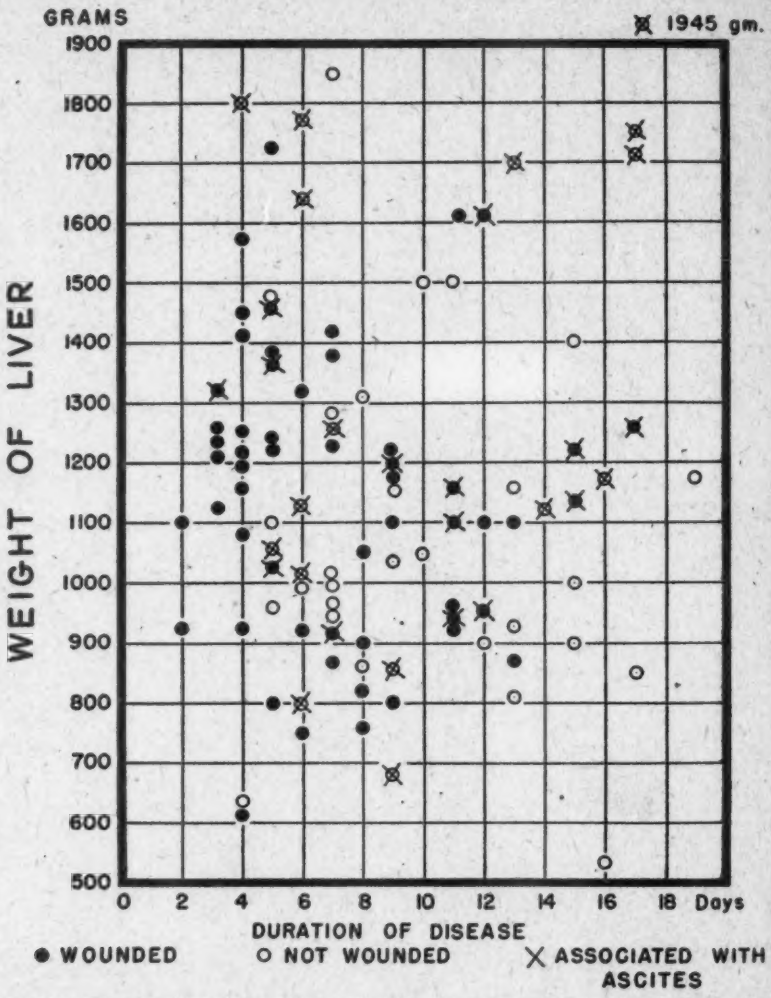
The differences in the incidence of ascites in the wounded and nonwounded groups are probably due to the more rapid course of the disease in the former.

SPLEEN

In approximately two-thirds of the cases the spleen was enlarged and its weight exceeded 200 gm.; in about one-fourth it weighed more than 300 gm. (Table XIII).

Detailed information pertaining to the relation of weight to duration for the wounded and nonwounded groups is given in Text-Figure 8.

It is clear that in these fulminant cases there is no definite relation of weights either to duration of illness or to the epidemiologic forms of hepatitis. For example, in the 6 cases in which death occurred 3 days



Text-Fig. 7. Distribution of hepatic weights in respect to duration of disease for wounded and nonwounded patients.

after onset of symptoms, 3 of the spleens weighed approximately 450 gm., the 3 others less than one-half as much. The somewhat greater number of large spleens among the wounded probably is relative to the preponderance of this group.

Grossly, the spleens in the fulminant cases usually had a tense capsule, but when bisected were found to be soft. The cut surface was dark red and congested. The follicles were large and in many instances contained a central opaque fleck.

Microscopically, the majority presented a well marked hyperplasia of reticulo-endothelial and lymphoid components. The follicles usually were prominent and their germinal centers were enlarged and often degenerated or necrotic (Fig. 27). Similar small focal areas of necrosis were frequently observed in the pulp. Occasionally the organ was conspicuously infiltrated with eosinophils and plasma cells. Nearly all spleens were greatly congested, often to such an extent that small

TABLE XIII

Weight of Spleen in 94 Cases of Fulminant Hepatitis

(gm.)	Number of cases
To 99	2
100—199	28
200—299	43
300—399	7
400—499	9
Over 500	5

hemorrhages had occurred. Rigidity of the sinuses with depletion of pulp, such as was the rule in the subacute cases, was not observed.

The common enlargement of the spleen in fulminant hepatitis is due to hyperplasia of its component cells and to acute congestion.

INTESTINES

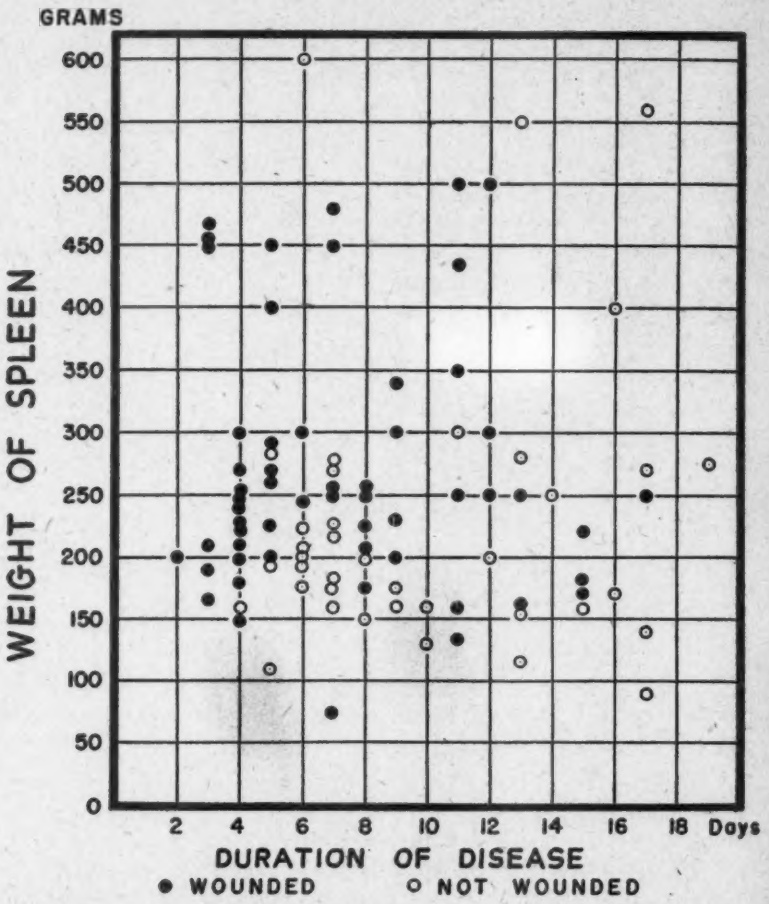
Phlegmonous inflammation of the colon, which occurred in 15 per cent of the subacute cases,[24] was not present in any fulminant ones. Occasionally there was a moderate degree of edema, always associated with ascites. Mucosal hemorrhages were equally as extensive as in the earlier series.

KIDNEYS

The kidneys were slightly swollen in most cases, 20 per cent being of normal size, 40 per cent weighing from 350 to 400 gm., and the remaining 40 per cent slightly above 400 gm.; no enlargement over 500 gm. was recorded. The variation in color was wide; some kidneys were dark from congestion; others were pale. In very few was any bile discoloration evident, and then it was confined to tinting of the pelvic epithelium; none was deeply bile-stained.

Microscopically, as observed in routine paraffin sections, there were few conspicuous changes. The glomeruli were uniformly normal except

for the presence in some instances of albuminous precipitates in the capsular spaces. The epithelium of the proximal convoluted tubules was generally slightly swollen and frequently showed basal vacuolization; the nuclei were well preserved and normally chromatic. Very



Text-Fig. 8. Distribution of splenic weights in respect to duration of disease for wounded and nonwounded patients.

little bile staining could be recognized in the epithelial cells, and bile-stained casts were seldom found. No severe degenerative changes and no necrosis were present at any level of the tubules. There was no interstitial reaction.

Fat deposits were usually conspicuous when frozen sections were stained with sudan stains. They were most marked in the proximal convoluted tubules (Figs. 28 and 29), beginning in the neck and con-

tinuing through the entire length; were less constant and less severe in the ascending limbs of Henle (Fig. 30), and still less so in the distal convoluted tubules. The deposits were always most apparent in the basal portion of the cells; the individual droplets were generally minute, but some attained the diameter of the nucleus. No fat was seen in the glomeruli, in the descending limbs of Henle, in the collecting tubules, or in the interstitial tissues. With polarized light the fat did not prove doubly refractile.

The degree of deposition of fat was estimated on a 1 to 3 plus scale in 39 cases chosen at random. No relation was found between its intensity and the degree of nitrogen retention, but when the amount of fat vacuolization was compared with duration of disease a distinct relationship became apparent. In 23 cases showing heavy deposits the average duration of hepatitis was 6 days. No fat, or only traces of it, was observed in 16 cases in which the average survival period was 11 days. Either the process is more severe when hepatitis is most fulminant, or it is a transitory phenomenon which tends to disappear with the passage of time. The lack of other evidences of epithelial degeneration suggests that it is a storage phenomenon, possibly dependent on the sudden liberation of large amounts of fat from the destroyed liver cells.

The absence or mildness of the degeneration observed in this series contrasts sharply with the severe grades of bile nephrosis noted in the subacute cases previously reported.[24]

BRAIN

The brain grossly presented no significant changes. Particularly noteworthy was the absence of hemorrhage. Microscopically, a nonspecific encephalopathy of mild degree was nearly always observed. In the most fulminant cases the bodies of many ganglion cells were swollen, the nuclei distorted and their chromatin granules dispersed and scattered. When the disease lasted more than 10 days some ganglion cells usually were shrunken and had dense pyknotic nuclei. Satellitosis and neuronophagy were moderate in some locations, but nowhere were they conspicuous. No perivascular round cell infiltrations, such as in the subacute form, were observed either in the meninges or the nervous system proper.

The glia presented interesting alterations. "Naked nuclei" were frequently numerous; they were poorly chromatic, somewhat swollen, and often slightly distorted. Nuclei of this kind have been described in many forms of hepatic disease; they are, however, not specific. The glial reaction was most evident in the basal ganglia.

Despite the prominence of nervous manifestations in the terminal

stage of hepatitis, the histologic changes usually are not impressive. This has been attributed to the tempo of the disease, for cerebral manifestations as a rule precede death by but few days.[41]

Clinicopathologic Correlations and Discussion

Fulminant hepatitis differs in numerous features from the subacute form which, until 2 or 3 years ago, had prevailed in all recorded epidemics. Is it a different disease? Are particular etiologic or epidemiologic factors responsible for its fulminant character? What is the mechanism of jaundice and of ascites in this form? What effect does rapid destruction of the liver have on the kidney? These are the questions we have selected for brief discussion. None can adequately be answered; rather, discussion can only indicate the need for further investigation.

Clinical and Epidemiologic Forms of Epidemic Hepatitis

Clinically, the fulminant form of hepatitis is characterized by the short and stormy course, the frequency of fever during the prodromal stage, and the brevity of the icteric stage. Biochemically, the fall in blood sugar and the low value of blood urea nitrogen reflect rapid and massive necrosis of the hepatic parenchyma. Pathologically, the outstanding lesions are extensive and comprise uniform destruction of liver cells, minimal evidence of regeneration, and marked inflammatory infiltration especially of the portal areas and the perilobular boundaries.

Consideration must be given the possibility that we are dealing with a disease different from epidemic hepatitis. Against this hypothesis two arguments can be raised. First, the similarities are greater than the differences, and these differences, in turn, are quantitative rather than qualitative. No new features, either clinical or pathological, have appeared in the current form, although some have been exaggerated or minimized. Second, all grades of transition between the fulminant and subacute forms have been observed. The conclusion is warranted, therefore, that fulminant hepatitis is a form of epidemic hepatitis, although final proof must wait upon advances in the determination of etiologic factors and upon controlled experimentation.

The specific causal agent of epidemic hepatitis has the attributes of a filtrable virus.[28,37,42–45] The virus has not as yet been cultivated, nor has any animal susceptible to it been found.[5,43] At present the only means of demonstrating the virus is by human transmission; in recent years this has been accomplished with volunteers.[33,42,44,46] The infectious agent has been shown to exist in the blood, the feces, the urine,

and nasal washings. Under natural conditions the disease probably is transmitted either through the alimentary tract by ingestion of contaminated food or water, or through the nasopharynx as droplet infection.[5,28,44] Under artificial conditions it may be transmitted by parenteral injection of blood, plasma, or serum from an individual harboring the virus.

Two main epidemiologic variants of the disease may be recognized: naturally occurring hepatitis (in endemic or epidemic form), and inoculation hepatitis. The latter may develop after transfusions of blood or its derivatives (homologous serum hepatitis),[5,27,29,30,32,42,54] after inoculation of vaccine containing small amounts of infectious serum (post-vaccinial hepatitis),[5,11,47,48] after injections of therapeutic agents through needles or syringes contaminated with blood from an infected person (hepatitis after insulin injection, post-arsphenamine hepatitis).[49–51] Transmission experiments indicate that the specific causal agents of these variants are similar or closely related.[33,37,42,45,46]

From the epidemiologic point of view the present series, regardless of duration, comprises 57 per cent of spontaneous (*i.e.*, naturally-occurring) cases (of which 16 per cent are regarded as endemic and 41 per cent as epidemic), and 43 per cent are believed to represent homologous serum hepatitis. Cases of the controversial post-arsphenamine hepatitis have been excluded, and there are no examples of post-vaccinial hepatitis. This study has shown that in the fulminant form the manifestations of the disease and the lesions of spontaneous and transfusion hepatitis are in every respect indistinguishable. Similarily, the less fulminant examples of this series present pathologic changes indistinguishable from those observed in cases of comparable duration in the 1942 epidemic (in which the majority could be regarded as post-vaccinial hepatitis), and from those reported in the Swedish epidemic of 1927 (which represented the naturally-occurring disease). These findings are in agreement with clinical observations [5,29,31,32,50,51] and with biopsy studies of nonfatal cases.[19,20,52]

It is concluded that the epidemiologic variants of the disease do not differ in their clinical manifestations.

Factors Responsible for the Fulminant Form of Fatal Hepatitis

Epidemic hepatitis is the only disease of this war which has become pandemic. With widespread prevalence there are indications that the disease has become more severe; thus many observers have reported that in a high proportion of cases, instead of the common insidious onset, the symptoms are those of an acute febrile infectious disease. Yet the mortality rate, so far as can be judged from figures at present

available, has not significantly altered. However, the course of fatal hepatitis has been more fulminant during the past 2 or 3 years. To what this may be attributed—whether to host factors, which have promoted an increase in individual susceptibility, or to changes in the infectious agent—is difficult to assess. It is not known whether a fulminant course is associated with an especially virulent strain or an especially large dose of virus. As to host factors which may increase susceptibility to the infection, either in fatal or nonfatal cases, a number have been suggested: age, physical strain, diet, other diseases,[5] combat fatigue,[8,12] accidental variation in resistance,[2] and a poor nutritional state.[8] In the present series we may at least rule out the factors of age, climate, or other variables depending on geographic location. All the other factors may well have played a part. They are interrelated; the most tangible ones are the effects of trauma and consequent transfusions, and the effects of nutritional disturbances. Even if we cannot, at this time, arrive at the correct evaluation of these factors, a discussion of them may point the way to future inquiry.

Trauma and Transfusion

Ninety per cent of the cases in this series occurred among combat troops in overseas theaters of war. Of the fulminant group nearly two-thirds (61 per cent) had sustained severe battle injuries or extensive burns; in sharp contrast, only 2 per cent of the group which survived for more than 20 days had been injured. Practically all casualties may be assumed to have received transfusions of blood or its derivatives. It is thus possible that the specific agent of hepatitis was transmitted directly and perhaps in large amounts. But tens of thousands of other men have sustained equally severe wounds, and have received transfusions wthout developing hepatitis. There is no accurate information as to how often hepatitis did develop. We do know that during the period of this study, covering nearly 2 years, the disease was fatal in less than 100 individuals who had been wounded and transfused.

Trauma, directly, cannot be implicated as the cause of fulminant hepatitis, but it may be important because it lowers individual host resistance in some manner.

The evidence that an infectious agent was transmitted by means of transfusion is circumstantial. It rests upon the well documented fact that the interval between parenteral introduction of virus and outbreak of symptoms, for all types of inoculation hepatitis, usually falls within a period of from 2 to 4 months.[27,29,37,42,47,48] The incubation period for the naturally-occurring disease is much shorter, and on the average

runs from 20 to 40 days.[37,42,45] The intervals between trauma and onset of symptoms in the present series is shown for 64 cases of fulminant hepatitis in Table XIV. It is a safe assumption that, for the majority of the casualties, plasma and transfusion therapy was concentrated within a few days of injury. Six cases of burns have not been included in this tabulation since these patients received plasma and whole blood for many weeks. From inspection of Table XIV it is evident that in the majority (69 per cent) the interval between transfusion therapy and onset of the disease fell within the limits of 50 and 89 days, the median duration being 70 days. It is very prob-

TABLE XIV

Interval between Receiving Wound and Initial Symptoms in 64 Cases of Fulminant Hepatitis

Interval	Number of cases	Per cent of cases
(*Days*)		
30—39	4	6
40—49	4	6
50—59	11	17
60—69	11	17
70—79	10	16
80—89	12	19
90—99	6	9
100—109	4	6
Over—110	2	3

able, therefore, that in these cases we are dealing with examples of hepatitis in which the virus was transmitted parenterally and not by "natural" means.

One distinct difference between naturally-occurring hepatitis and serum hepatitis in this series is the duration of disease; the course of the serum type tends to be more rapid (see Tables II and IV and Text-Figs. 1 to 3). Whether this is a general characteristic of this type of hepatitis cannot yet be stated with certainty; it may depend on the particular strains of virus injected, and on nonspecific individual host factors incident to combat trauma. In this connection the classical experiments of Opie [58] may be recalled. He demonstrated that the activity of a hepatic poison may be so enhanced by bacterial infection that a quantity of the poison which alone produces little change may, in combination with infective agents, cause destruction of almost the entire hepatic parenchyma. Opie believed it probable that those instances of acute yellow atrophy which accompany streptococcic infections are dependent upon some disturbance of metabolism or other form of intoxication which renders the liver unusually susceptible. Pertinent, too, are experiments of Bennett, Drinker, and Warren [55] on the effects of certain chlorinated hydrocarbons which may produce

extensive necrosis of the liver. These compounds are extensively used in industry, but the incidence of "acute yellow atrophy" in workers exposed to the compounds is very low. This fact suggests that certain individuals may be more susceptible, or that in fatal cases liver damage may have been augumented by some other agent. The latter hypothesis was tested by experiments on rats with confirmatory results; the effect of the compounds under discussion was found to be much enhanced by small sublethal doses of other hepatic poisons.

Nutritional Disturbances

It is well known that liver injury may be produced by purely nutritional factors.[56,57] Several observers have implicated improper or inadequate diet and poor nutritional states as increasing susceptibility to the infectious agent of hepatitis.[3,5] What actual part such factors have played in the present series cannot be determined. Even on an adequate Army diet, some soldiers lose appetite and weight; it may be assumed that the strain and the exigencies of warfare have brought about very considerable disturbances of the nutritional state, and thus, perhaps, of resistance to the infectious agent. The recent experiments of Glynn and Himsworth[58,59] have great interest in relation to this problem. These investigators demonstrated that massive necrosis may be produced in rats without the intervention of any exogenous toxic agent, chemical or infective, solely as the result of a deficient diet. The essential factor in which it is deficient appears to be an amino acid. The destruction of hepatic parenchyma in these rats has many resemblances to that of fatal epidemic hepatitis in man. It develops abruptly after a latent period of several weeks, during which the liver is morphologically normal. The latent period probably represents the time required to deplete the reserve of certain essential constituents in the body's store of labile proteins. The lesions appear in two forms: a generalized massive necrosis when the diet is grossly deficient in necessary protein constituents, or a necrosis confined to certain parts of the liver, especially the left lobe, when the diet is less deficient but still not adequate.

Mechanism of Jaundice

In the previous study of hepatitis[24] the general mechanism of jaundice has been discussed. Here it remains to inquire whether the same mechanism is operating in the rapidly destructive fulminant form of the disease.

In the subacute form of hepatitis the cause of jaundice appears to be principally a mechanical obstruction by bile "thrombi" of the

intralobular canaliculi in the surviving or regenerating liver tissue. Similarly, in liver taken for biopsy from nonfatal cases the canaliculi are occluded by plugs of altered and inspissated bile.[40] In fulminant hepatitis destruction of liver cells is often complete; at best only small rims of peripheral cells are spared. Necrosis of the hepatic columns leads, of course, to destruction of the delicate bile clefts, the canaliculi; obstructive factors cannot, therefore, be considered. Jaundice can only be accounted for by the rapid destruction of liver cells; the absence of liver cells makes it impossible to remove bilirubin from the circulating blood (or from the Kupffer cells).

But what of fulminant hepatitis without jaundice? This explanation gives no clue to the situation there. Careful study of the several examples in this series disclosed no differences in lesions. In examples included among representative cases (nos. 2, 5, and 7), the duration of the disease was 3, 4, and 6 days respectively, a sufficient time for jaundice to have appeared. No explanation can be offered for the absence of jaundice despite complete destruction of liver. Even though anicteric fatal hepatitis is the exception, it indicates the need for reinvestigation of the complex mechanism of jaundice.

Ascites

The mechanism of ascites is equally complex. It is surprising to find that approximately one-fourth of the fulminant cases are associated with ascites. Its incidence is definitely correlated with duration of disease, rising to 70 per cent in subacute hepatitis. This suggests that the factors which lead to the accumulation of fluid become more pronounced as the disease is protracted. There is also a correlation between ascites and weight of liver, in that ascites is most frequently associated with livers that are but little shrunken. As has been shown, the weight of the liver in the fulminant group is largely a function of its blood content. Destruction of the columns of liver cells tends to bring about dilation and congestion of the sinusoids, that is to say, stasis. But stasis does not invariably occur, nor is it always uniformly distributed throughout the liver. It is possible that such variations in blood content of the liver are due to alterations in the action of the diaphragm. This muscle renders an important aid to venous flow; it has been likened to a hand which rhythmically squeezes a sponge filled with blood.[60] Since clinical evidence shows that ascites develops usually during the terminal phase of the disease, more or less simultaneously with disturbances of the central nervous system, there exists the possibility that diaphragmatic innervation and action may be impaired. Whatever the rôle of the diaphragm may be, it seems plausible

that interference with venous escape from the liver is one of the chief causes of the ascites of fulminant hepatitis.

One other point deserves mention. In the experimental massive necrosis resulting from dietary deficiency, ascites commonly develops.[58] Mann and Bollman also have pointed out that ascites may be produced by dietary means.[56,57] Whether and to what extent such factors operate in fulminant hepatitis is problematical. Disturbances of plasma proteins resulting from destruction of liver, and their bearing on ascites cannot, in our series, be properly evaluated.

Fulminant Hepatitis and the Hepatorenal Syndrome

Does the massive and rapid disintegration of liver cells which is characteristic of fulminant hepatitis lead to the symptom complex known as the "hepatorenal syndrome"?

Heyd [61] is generally given credit for having focused attention, in 1924, on certain types of "liver death" most commonly seen following surgical operations upon the biliary tract or severe trauma to the liver. His original description was amplified in subsequent reports [62] and his concept has been added to and modified by numerous other authors, notably Cave,[63] Helwig and associates,[64-66] Boyce and McFetridge,[67] and Wilensky.[68,69] Most recent authors have emphasized a renal as well as a hepatic element, and the term "hepatorenal syndrome" has become widely used, but has never been clearly defined. The present consensus, as summarized by Thorn,[70] distinguishes two symptom complexes: one characterized by sudden onset of hyperthermia, delirium and coma; the second by renal failure and uremic death, often preceded by an episode of circulatory collapse or other shock-like state. Evidence for a renal component in the first syndrome is minimal and consists of vaguely described, nonspecific changes of kidney structure, falling within the limits of "cloudy swelling," or of bile nephrosis in cases in which jaundice precedes the acute episode. In the second syndrome, degenerative changes of greater severity have usually, though not invariably, been recorded, but no constant picture emerges from the reports. At the time when they were published, neither clinicians nor pathologists were aware of the profound renal damage which may follow shock regardless of whether the liver is diseased or traumatized.

A sharp distinction must be made between the hepatorenal syndrome and bile, or cholemic, nephrosis. The former is ill defined, the latter well recognized.[71-73] Briefly stated, cholemic nephrosis is associated with prolonged jaundice; it occurred in most cases of subacute

hepatitis.[24] The essential lesions are tubular degeneration of varying severity, the presence of bile casts, and alterations in glomerular permeability. It is usually assumed, and experimental evidence supports the hypothesis,[74] that bile salts are injurious to renal epithelium and are the primary cause of degenerative damage. Since bile salts are formed in the liver, massive destruction of this organ would tend to lessen rather than to increase their excretion by the kidney. Clinical studies of renal function in jaundiced patients[75,76] likewise demonstrated renal damage and proved, furthermore, that it was ordinarily reversible since the albuminuria, cylindruria, and slight grades of azotemia cleared with disappearance of icterus. Nearly all authorities agree that simple bile nephrosis does not explain the severe renal insufficiency of the "hepatorenal syndrome."[65,74]

Two hypotheses have been repeatedly suggested to account for this syndrome: (a) that a toxic substance is elaborated by the sudden destruction of liver parenchyma, and (b) that a malfunctioning liver fails to detoxify an unknown nephrotoxic agent from some other source. If either of these hypotheses were correct, fulminant hepatitis with its massive and rapid destruction of liver parenchyma should provide many examples of the hepatorenal syndrome.

If one accepts a broad definition of this syndrome[68,69] and includes hyperthermic deaths and all forms and degrees of simultaneous or successive impairment of liver and kidney function, our data provide positive evidence for its existence in fulminant hepatitis since the majority of cases showed both clinical and histologic evidence of some renal injury. A definition so inclusive is, however, all but meaningless. If the more usual and narrower definition of the hepatorenal syndrome is accepted, namely, that of renal damage consequent to liver disease and so severe that it leads to uremic death, our data are essentially negative.

Pathologically, in the fulminant cases of hepatitis no significant renal changes other than storage of fat were observed and the decreasing degree of this phenomenon with longer periods of survival indicates that it was reversible and transitory in character. From the clinical point of view the only important evidence suggestive of renal insufficiency was azotemia, but in only 4 cases were nonprotein nitrogen levels above 60 mg. per cent recorded, with a maximal figure of 103 in one case. Azotemia of even higher levels would constitute inadequate proof of renal impairment in view of the rapid and extensive liberation of protein which must result from the massive necrosis of liver cells. There was no correlation between degree of fat storage

and nitrogen retention. It is concluded that fulminant hepatitis is not associated with the "hepatorenal syndrome" as this syndrome is usually defined.

Summary

A fulminant form of epidemic hepatitis which runs a fatal course in less than 10 days has appeared during the past 3 years. In a new series of 196 cases of fatal hepatitis which occurred in the U. S. Army between August, 1943, and April, 1945, approximately one-half (53 per cent) were of this type. By contrast, not a single such case was observed during the Army epidemic of 1942 and only one during the Swedish epidemic of 1927; then the median duration of fatal hepatitis exceeded 5 weeks. The clinical features and pathologic changes of the fulminant form differ significantly from those of the subacute variety which predominated in previous epidemics.

On the basis of epidemiology the present series includes 29 examples of the endemic and 72 of the epidemic variant of "spontaneous" hepatitis, and 77 cases presumed to be "homologous serum hepatitis" following trauma and transfusions of blood or blood derivatives. Analysis makes it evident that the epidemiologic type does not determine the clinical form of hepatitis, whether fulminant or more protracted.

This study is based principally on 94 cases in which the clinical course of the disease did not exceed 9 days. Thirty-nine others with a duration of from 10 to 19 days have been used to supply additional information, for many of them had lesions indistinguishable from those of the more fulminant form. The remainder of the series, *i.e.*, the subacute cases, which clinically and pathologically resemble those of the 1942 epidemic, are considered only in connection with certain analyses, such as the significance of geographic factors.

No precise information is available as to whether the mortality rates are the same or different in the several epidemiologic variants. The average mortality during the period covered by this study was 0.3 per cent. Serum hepatitis tended to run a considerably more rapid course than the naturally occurring disease, but otherwise there were no discernible differences, either clinical or pathologic, between these variants.

Clinically, fulminant hepatitis was characterized by a sharp and stormy course. It usually was ushered in by one of two syndromes: (1) an "infectious" type in which high fever, chilliness, malaise, and general aching dominated the picture, and (2) a "gastrointestinal" type with anorexia, nausea and epigastric discomfort in the foreground. These two types were represented in approximately equal proportions, and during various epidemics often occurred side by side. The subse-

quent clinical manifestations bore no relation to the prodromal symptoms. Because of the brevity of the course, the initial symptoms sometimes merged with those of the terminal stage.

Temperature records were available for 68 of the fulminant cases. In all but one the onset was febrile. The temperature ranged from 95° to 104° and averaged 102° F., fever declining as a rule with the onset of jaundice. During the final stage of the disease there was almost invariably a sharp rise in temperature coincident with profound cerebral disturbances.

In contrast to the deep jaundice commonly observed in the subacute form, the degree of jaundice in fulminant hepatitis was often mild. Several anicteric cases are included in this series.

Among noteworthy laboratory findings were moderate degrees of nitrogen retention and lowering of blood sugar.

Pathologically, lesions other than those in the liver were relatively slight; the changes found in "spontaneous" and in "inoculation" hepatitis were in every respect similar. The lesion of the liver was characterized by extreme and often complete destruction of hepatic cells, and by a marked inflammatory reaction. Typically, the involvement was uniform. The gross appearance of the liver was not pathognomonic and gave no indication either of the extent of parenchymatous destruction or of the degree of inflammatory infiltration. The organ usually was flaccid and moderately shrunken, and the capsule was smooth or finely wrinkled. The cut surface most often presented an exaggerated "nutmeg" pattern, though sometimes it resembled that of an acutely congested spleen.

Microscopically, the destructive process was limited specifically to liver cells. Even in the more rapidly fatal cases the earliest stages of cell disintegration could not be observed; the dead cells had undergone lysis and the resultant débris had already been removed. The inflammatory infiltration was most conspicuous at the lobular peripheries and less so within the lobular remnants. The predominating cells were mononuclear forms—reticulo-endothelial derivatives, plasma cells, and lymphocytes. Regenerative hyperplasia of surviving parenchyma was minimal and confined to biliary rather than to hepatic epithelium. There was often a marked disparity between the apparent age of the lesions and the duration of symptoms. The pathologic changes in the liver were, in many instances, obviously older than the clinical history suggested; less frequently the reverse was true.

The spleen usually showed acute congestion and hyperplasia of its component cells. Focal areas of necrosis were common in the follicles and in the pulp.

The kidneys in the majority of cases were the site of marked fat storage, especially within the cells of the proximal convoluted tubules. The storage was not associated with significant degenerative changes; it probably was the result of sudden liberation of large amounts of fat from the destroyed liver cells. There was no correlation between degree of fat storage and nitrogen retention. The rapid destruction of the hepatic parenchyma did not lead to the development of the "hepatorenal syndrome" as it is usually defined.

Despite the marked nervous disturbances in the terminal stage of hepatitis, histologic changes in the brain were usually slight and consisted of a mild nonspecific encephalopathy.

The mechanism of jaundice in fulminant hepatitis is complex. The extensive and often complete destruction of liver cells must be considered a chief cause. No adequate explanation can be offered for the occasional occurrence of entirely anicteric cases of fulminant hepatitis.

Ascites was present in approximately one-fourth of the cases of fulminant hepatitis. The principal factor in its production is believed to be acute venous stasis in the liver.

The factors responsible for the appearance, during recent epidemics, of hepatitis in a fulminant form are difficult to assess. It is suggested that more or less interrelated host factors, such as fatigue, trauma and nutritional disturbances, rather than the strain or the amount of the infectious agent, play a dominant part.

We wish to express our grateful appreciation to the Staff of the Army Medical Library for the translation of a Russian article and for bibliographic assistance.

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[*Illustrations follow*]

DESCRIPTIONS OF PLATES

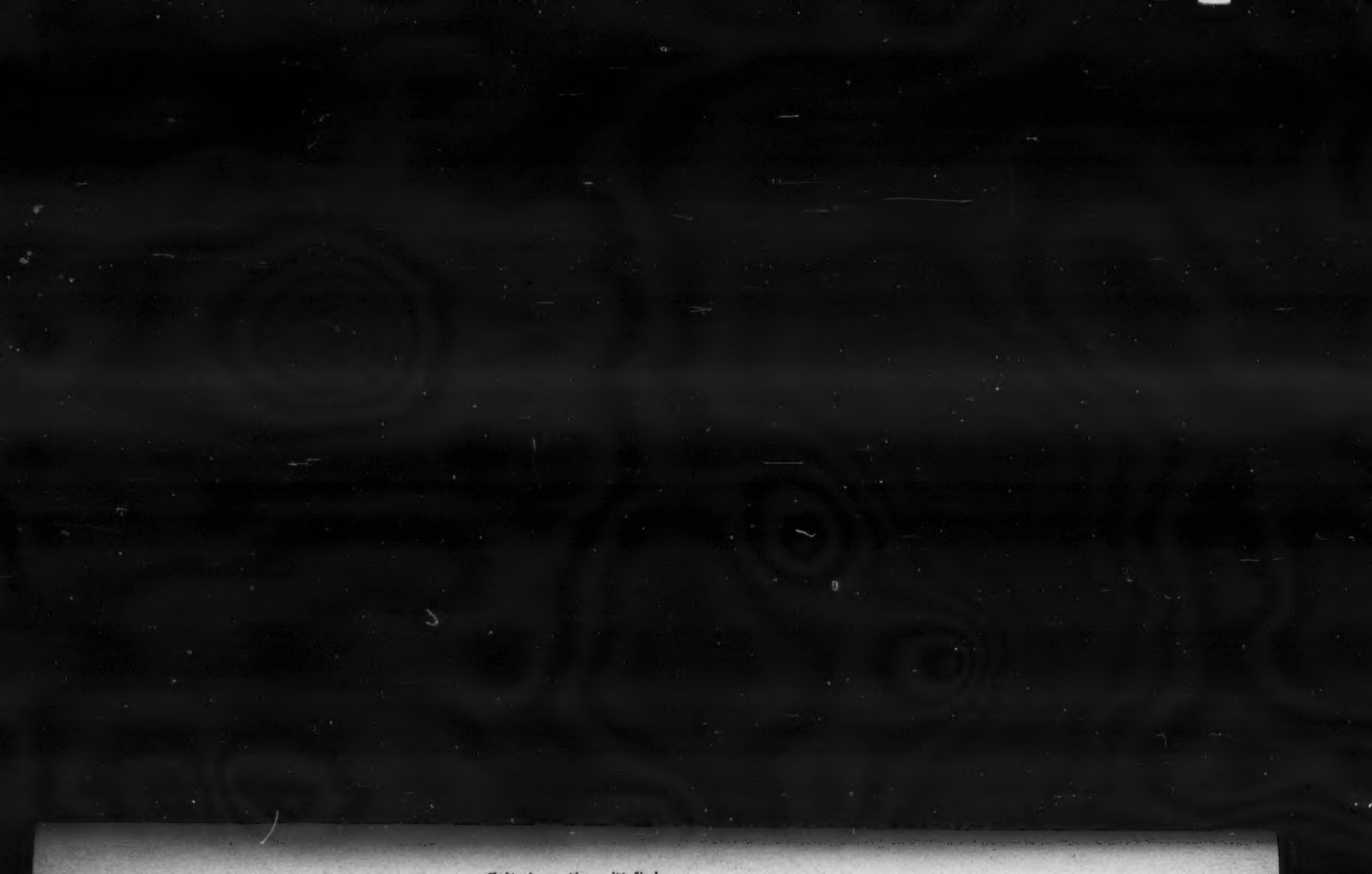
PLATE 164

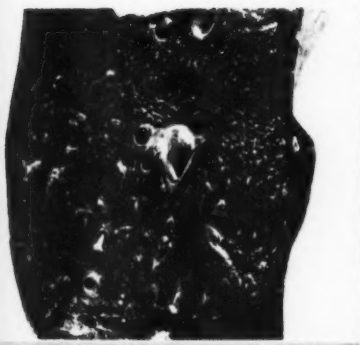
FIG. 1. Clinical duration of hepatitis, 4 days. Upper surface of the liver, which weighed 1200 gm. The surface of the right lobe is smooth; there are a number of subcapsular hemorrhages. The surface of the left lobe is finely wrinkled. (Army Institute of Pathology accession no. 148911.)

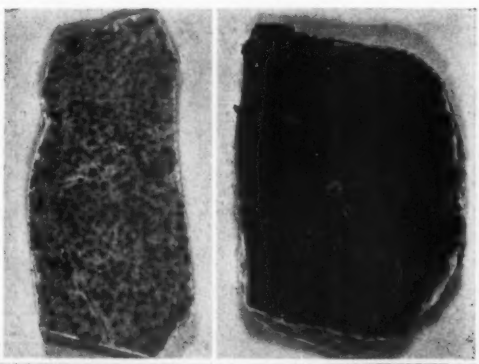
FIG. 2. Cut surface of liver shown in Figure 1. The appearance is similar to that of an acutely congested and hyperplastic spleen.

FIGS. 3 to 7. Representative areas of cut surfaces of livers from five cases of fulminant hepatitis; all have an exaggerated "nutmeg" mottling. Naked-eye examinations of these livers gave no indication of the extent of the parenchymatous destruction or of the prominence of inflammatory infiltration.

Fig.	Duration of disease	Weight of liver	Case no.
3	3 days	1205 gm.	126048
4	4 days	1200 gm.	126423
5	5 days	1025 gm.	128680
6	8 days	Normal size	103350
7	8 days	Shrunken	128577







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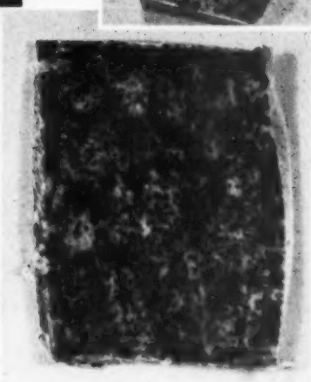


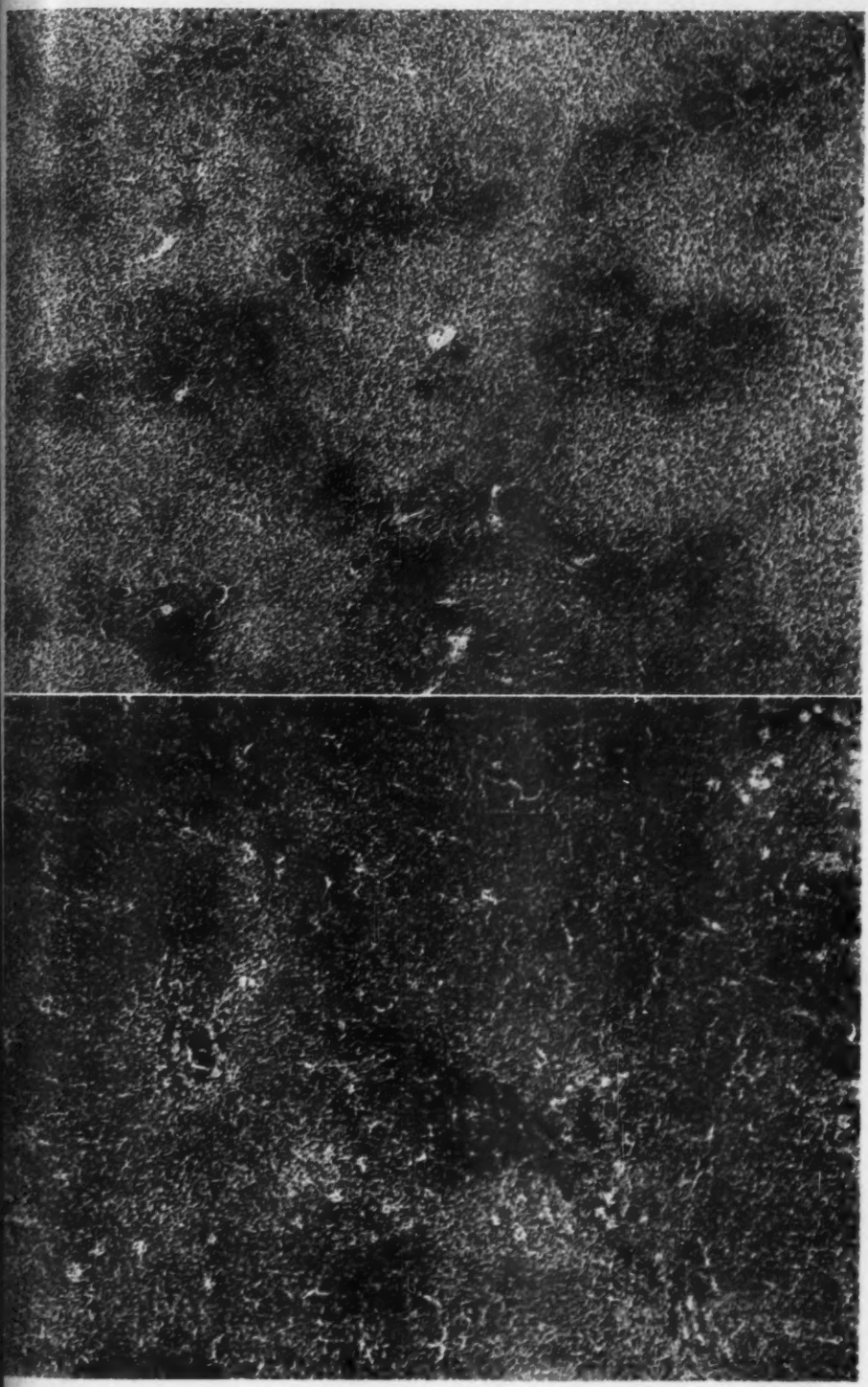
5 6 7

Lucké and Mallory The Fulminant Form of Epidemic Hepatitis

PLATE 165

FIG. 8. Duration of disease, clinically less than 1 day. (See case report 21.) Microscopic appearance of the liver at low magnification. The hepatic parenchyma has been destroyed. The portal regions and the perilobular boundaries are densely infiltrated with inflammatory cells. (See Fig. 15 for appearance of a lobule at higher magnification, Fig. 24 for details of the inflammatory reaction, and Fig. 27 for changes in spleen.) × 25. (A.I.P. acc. 123465.)

FIG. 9. Duration of disease, 3 days. (See case report 1.) Photomicrograph of liver at low magnification. The hepatic cells have been destroyed. The lobular remnants are engorged with blood. The peripheries are outlined by bands of inflammatory cells. (Fig. 10 shows a portal region at higher magnification, Fig. 12 details of the cells, and Fig. 19 illustrates the observation that in areas of parenchymatous destruction the reticulum framework is preserved.) × 25. (A.I.P. acc. 124057.)



Lucké and Mallory The Fulminant Form of Epidemic Hepatitis

PLATE 166

FIG. 10. Duration of disease, 3 days. (See case 1.) Cell infiltration of the portal stroma at higher magnification than in the preceding photomicrograph. In the upper right-hand corner of the figure is shown the complete destruction of hepatic cells and great engorgement of the lobular remnants. Several bile ducts are sending out solid buds of proliferative epithelium. (See also Figs. 9, 12, and 19). × 400. (A.I.P. acc. 124057.)

FIG. 11. Duration of disease, 9 days. (See case 13.) A representative picture of the cellular composition of the infiltrate at the lobular peripheries. Most of the cells are large mononuclears, but some plasma cells and lymphocytes are also present. × 900. (A.I.P. acc. 114947.)

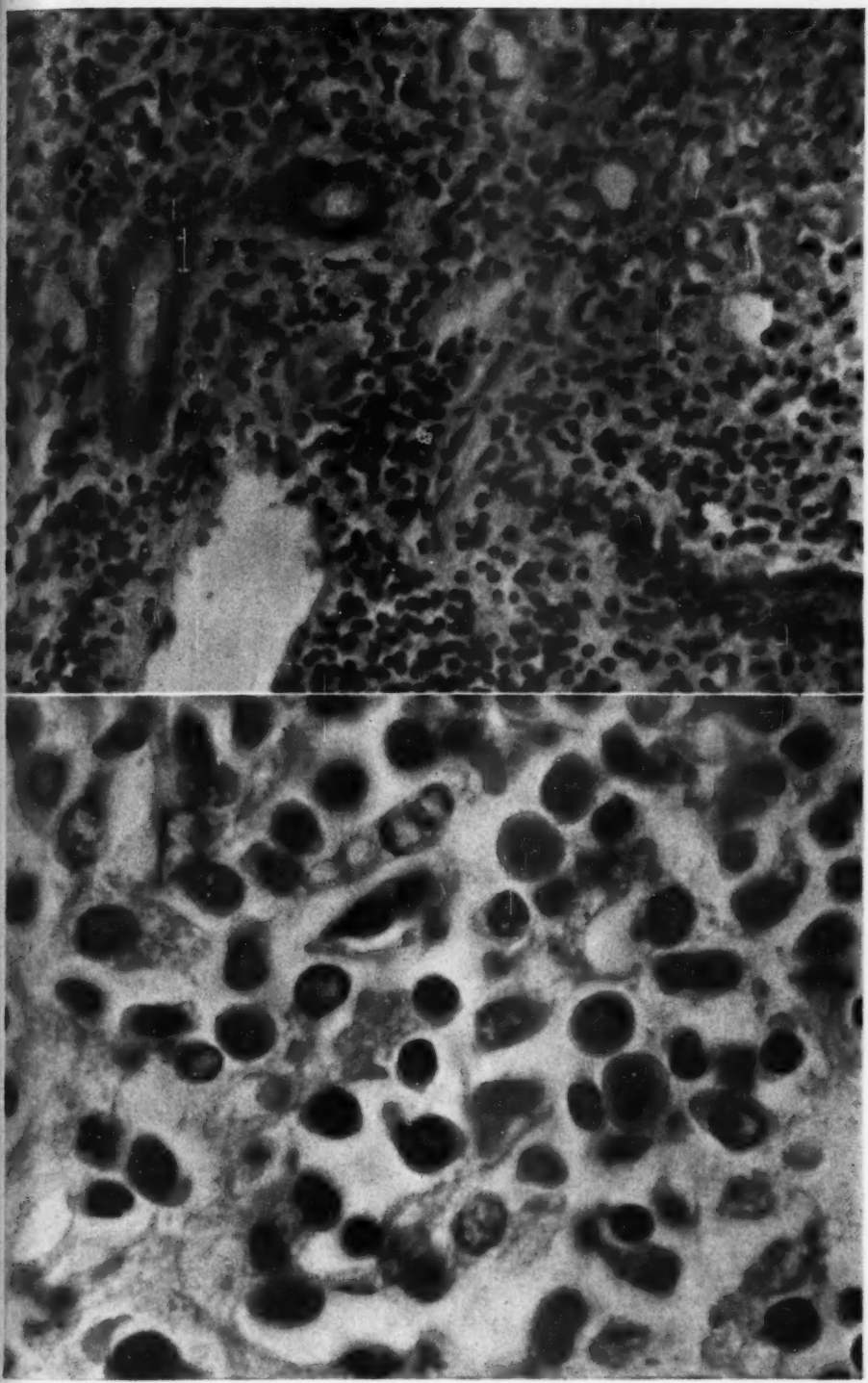


PLATE 167

FIG. 12. Duration of disease, 3 days. (See case 1.) The figure shows a group of plasma cells. In many cases these cells are numerically conspicuous, although they rarely predominate. (See also Figs. 9, 10, and 19.) × 2280. (A.I.P. acc. 124057.)

FIG. 13. Duration of disease, 7 days. Several eosinophils may be seen among the cells at a lobular periphery. × 1300. (A.I.P. acc. 133951.)

FIG. 14. Duration of disease, 9 days. Cell reaction within the lobular remnants. The predominant cells are mobilized and proliferated macrophages; many have ingested a brownish pigment, lipofuscin. × 1300. (A.I.P. acc. 131876.)

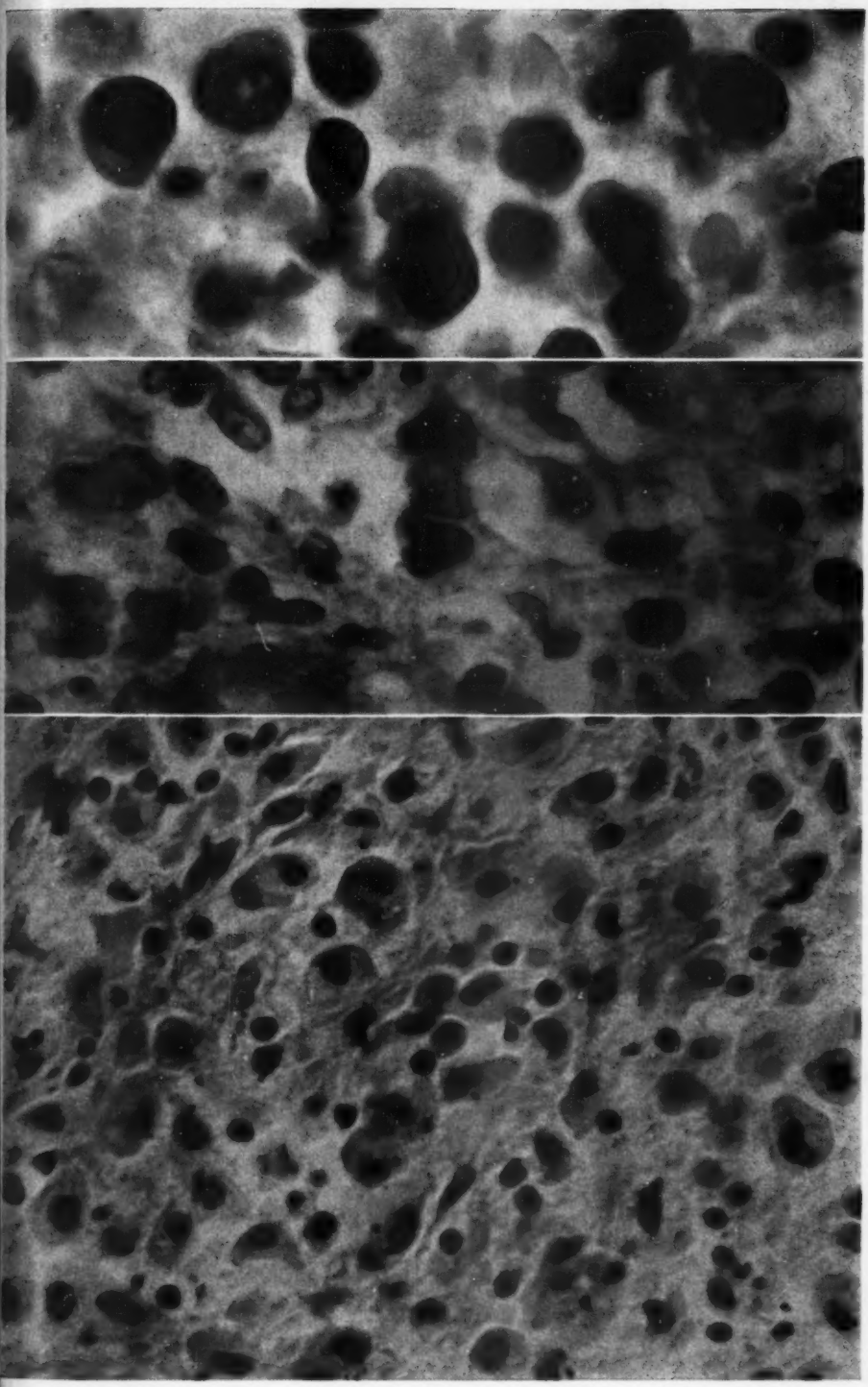
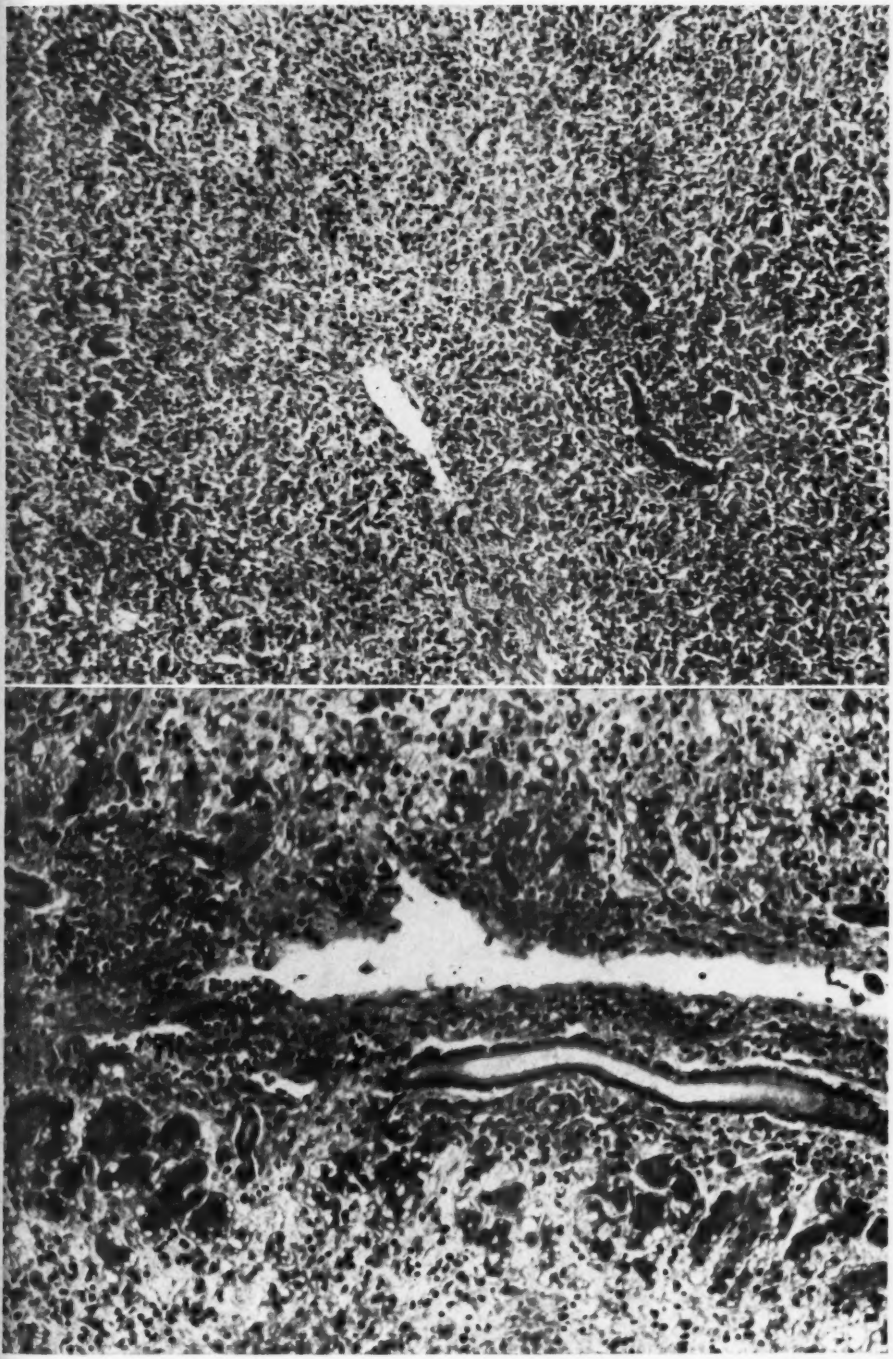


PLATE 168

FIG. 15. Duration of disease, clinically less than 1 day. (See case report 21.) A hepatic lobule at higher magnification than shown in Figure 8. In the photomicrograph the central lobular vein may easily be recognized; the lobular boundaries are indicated by small proliferating bile ducts, the significance of which is discussed in the text. The hepatic parenchyma has been destroyed; the lobular remnants are invaded by inflammatory cells, the nuclei of which appear as black dots. (See also Figs. 8, 24, and 27.) × 145. (A.I.P. acc. 123465; neg. 86343.)

FIG. 16. Duration of disease, 4 days. (See case report 17.) The figure shows a portal region with vein and bile duct cut longitudinally. The stroma is infiltrated with mononuclear cells which extend into the interior of the adjacent lobules. Isolated liver cells remain at the extreme periphery of the lobule; the great bulk of the parenchyma is destroyed. × 175. (A.I.P. acc. 129051; neg. 86333.)

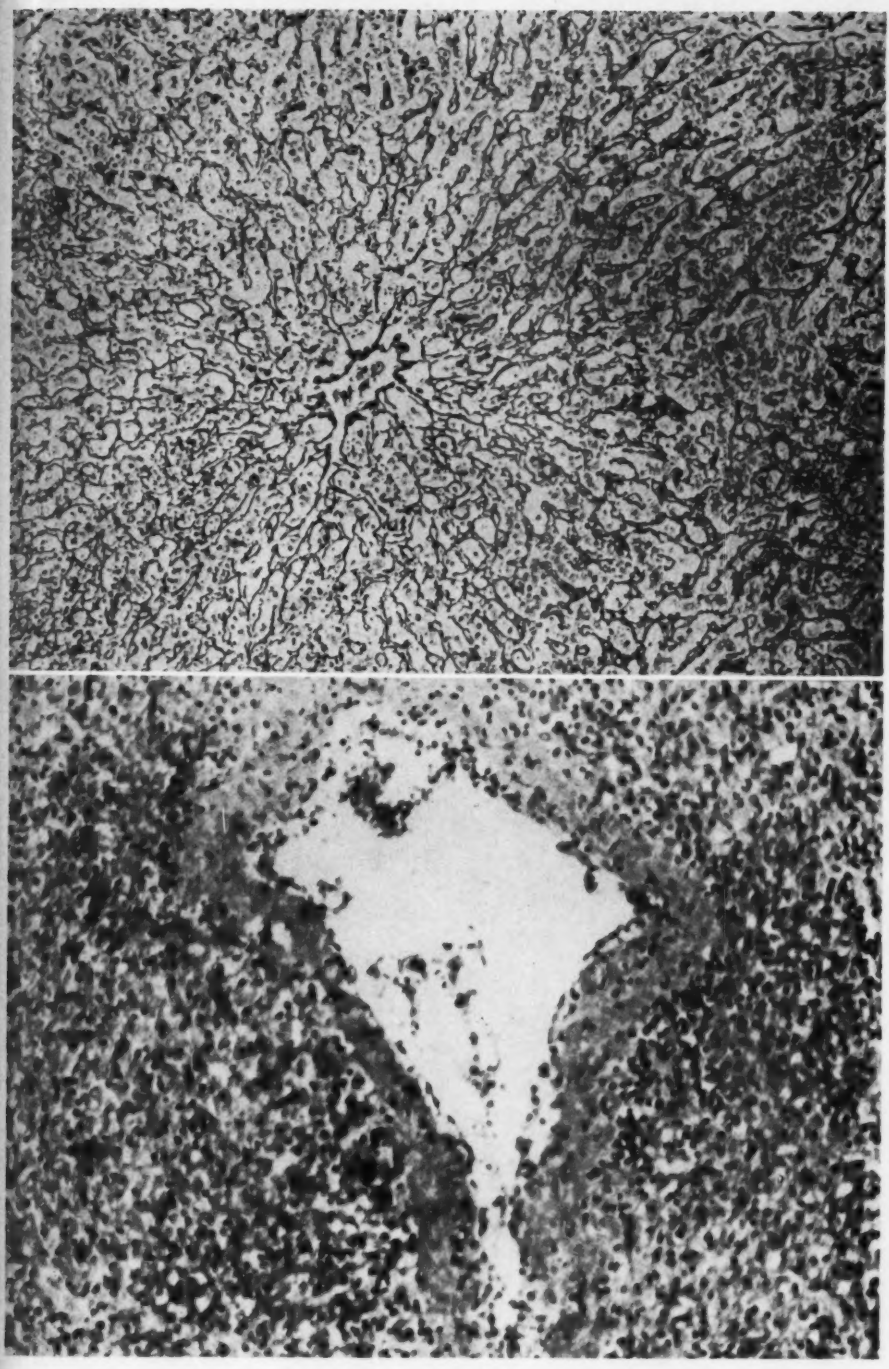


Lucké and Mallory The Fulminant Form of Epidemic Hepatitis

PLATE 169

FIG. 17. Duration of disease, 3 days. (See case report 2.) The figure shows the intact reticulum frame of a lobule, the parenchyma of which has been completely destroyed. (See Fig. 3 for gross appearance of cut surface of liver; Fig. 18 for change in central lobular vein, and Fig. 23 for proliferation of septal bile ducts.) Wilder's reticulum stain. × 145. (A.I.P. acc. 126048; neg. 86345.)

FIG. 18. Duration of disease, 3 days. (See case report 2.) A central lobular vein has a conspicuously thickened wall which appears hyalinized, and is invaded by a few inflammatory cells. The surrounding tissue is congested and infiltrated with mononuclear cells. The hepatic parenchyma has been destroyed. (See Fig. 17 which shows that the reticulum framework is preserved.) × 205. (A.I.P. acc. 126048; neg. 86344.)



Lucké and Mallory The Fulminant Form of Epidemic Hepatitis

Plate 170

Fig. 19. Duration of disease, 3 days. (See case report 1.) A hepatic lobule, the periphery of which is densely infiltrated with inflammatory cells which extend into the interior of the lobule. The hepatic parenchyma has been entirely destroyed and traces of liver cells have disappeared. The reticulum framework, however, was preserved. The lobular remnants are greatly engorged with blood. (See Fig. 9 for a photomicrograph in color showing a larger field at lower magnification, and Figs. 10 and 12 for cellular details.) × 160. (A.I.P. acc. 124057; neg. 86346.)

Fig. 20. Duration of disease, 8 days. A portal triad at the junction of three adjacent lobules. There is a conspicuous inflammatory reaction which extends along the twigs of the bile ducts and vessels into the perilobular boundaries. The liver cells have disappeared. (See Fig. 7 for gross appearance of cut surface of liver.) × 145. (A.I.P. acc. 128577; neg. 86336.)

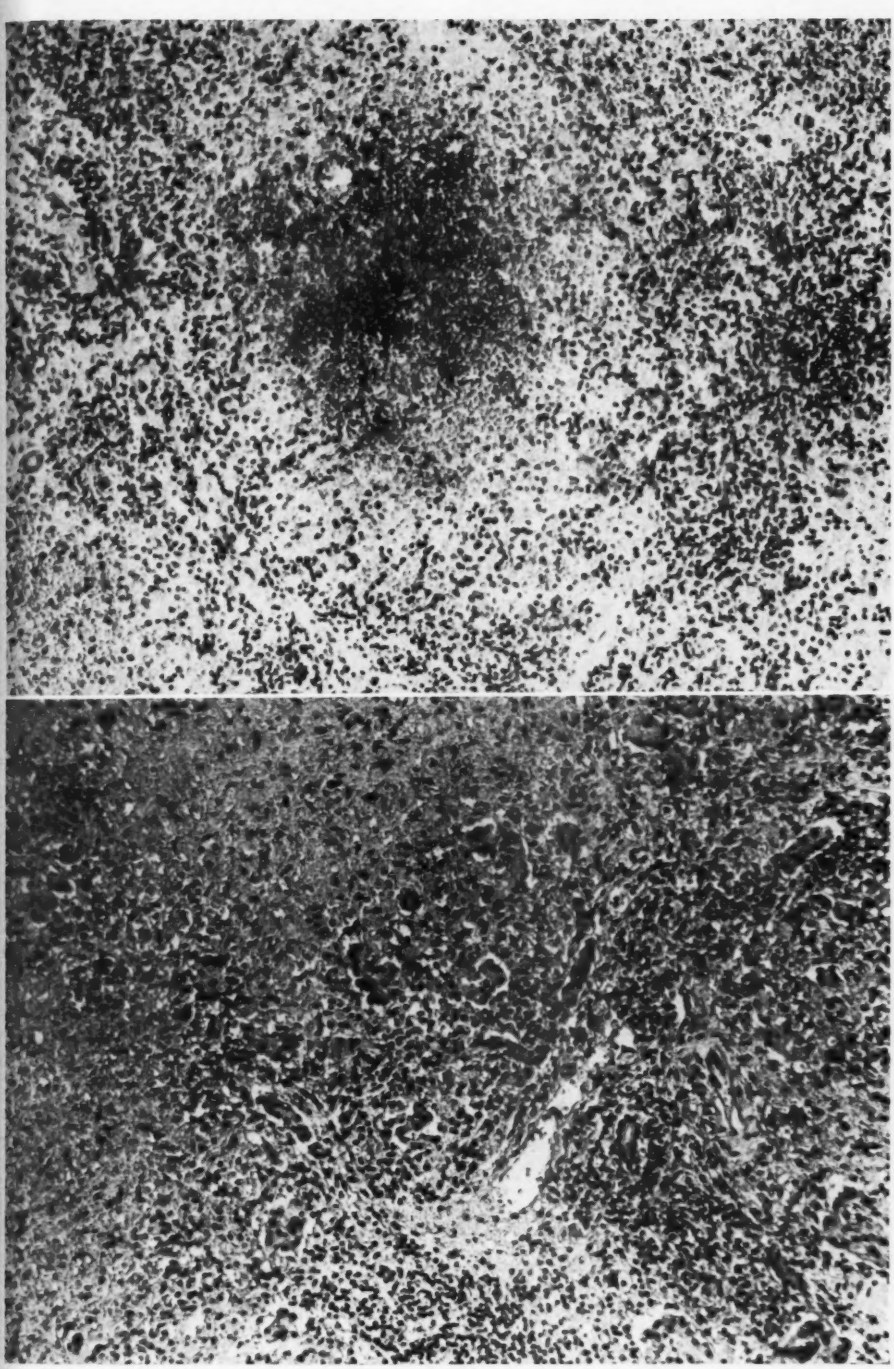


PLATE 171

FIG. 21. Duration of disease, 7 days. Inflammatory reaction within a lobule. The parenchymatous cells have been destroyed and their débris has been removed. The section has been stained by Masson's trichrome method to bring out the boundaries of the sinusoids, and thus to show the distribution of the inflammatory cells. It will be noted, first, that the sinusoids are widely dilated (due to the loss of the hepatic columns); second, that the inflammatory cells (mostly mononuclear) lie both within and between the sinusoids; and third, that because of these changes, the hepatic tissue resembles splenic pulp. The dark appearance of some histiocytes is due to ingestion of a pigment, lipofuscin. × 600. (A.I.P. acc. 126143; neg. 86326.)

FIG. 22. Duration of disease, 7 days. The figure shows a field near the periphery of a lobule. The parenchyma has been destroyed; the vascular stroma is invaded by plasma cells. The large coherent cells in the upper left of the photograph probably are proliferating biliary epithelium. In the upper center may be seen a congested sinusoid with intact walls. × 1360. (A.I.P. acc. 133195; neg. 86342.)

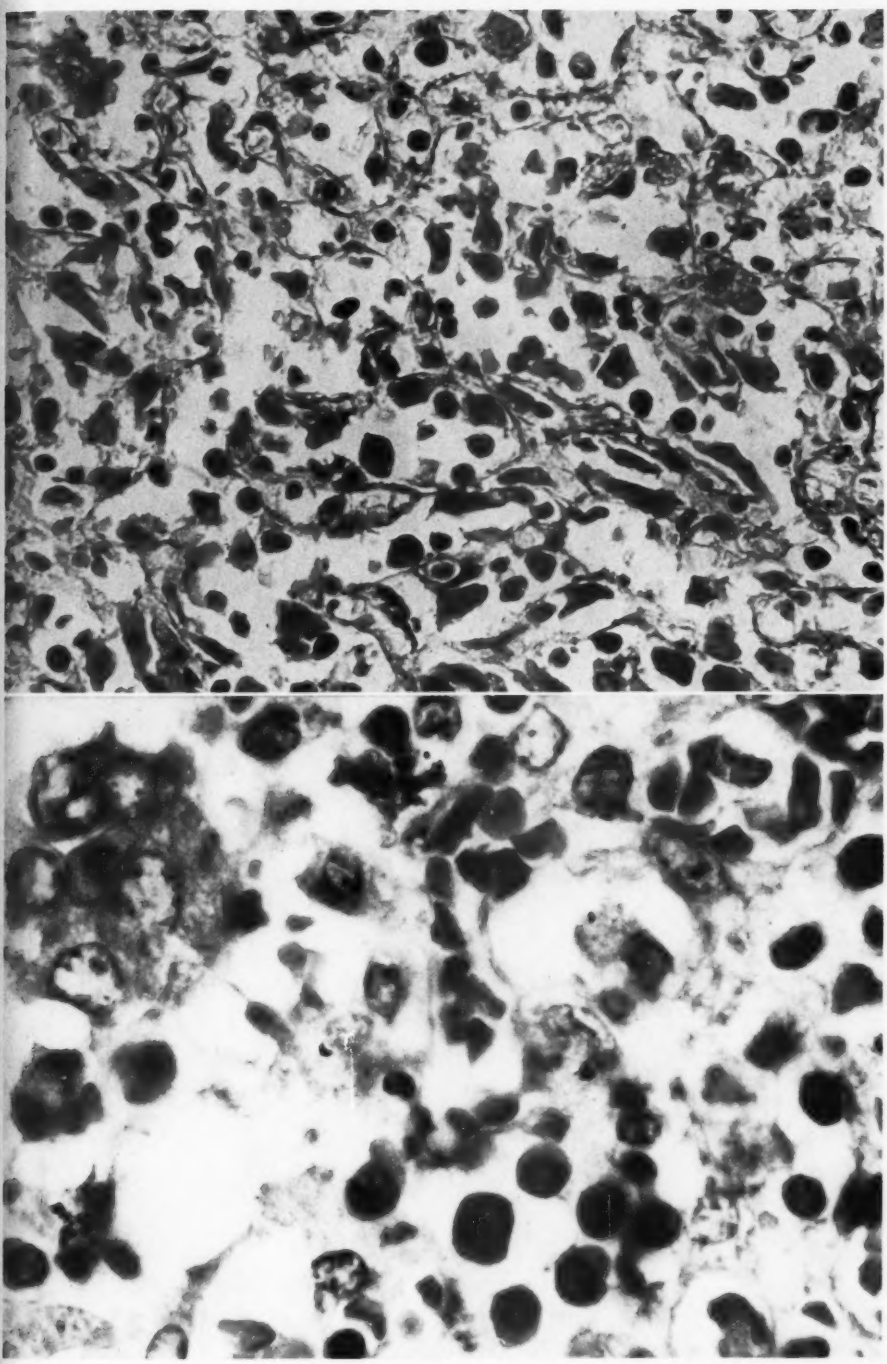


PLATE 172

FIG. 23. Duration of disease, 3 days. (See case report 2.) Proliferation of septal (perilobular) bile ducts at the boundary of two adjacent lobules. These little ducts are normally inconspicuous and resemble vascular twigs. Here, their cells are prominent, crowded together, and have large hyperchromatic nuclei. In the upper half of the photograph are shown several sinusoids with intact walls. The intervening hepatic cells have disappeared, and the lobular remnants are greatly engorged. (See also Figs. 3, 17, and 18.) × 650. (A.I.P. acc. 126048; neg. 86332.)

FIG. 24. Duration of disease, clinically less than 1 day. (See case report 21.) Portal region. A proliferating bile duct, composed of large cells with prominent nuclei, lies diagonally across the photograph. On one side of the duct the stroma is densely infiltrated with mononuclear cells, among which may be seen a few polymorphonuclear leukocytes. The large pale cells on the opposite side of the duct are swollen histiocytes. (See also Figs. 8, 15, and 27.) × 915. (A.I.P. acc. 123465; neg. 86347.)

FIG. 25. Duration of disease, 9 days. Inflammatory cells in the portal stroma: plasma cells, lymphocytes, and histiocytes predominate. The mass in the upper left-hand corner consists of proliferating bile duct epithelium. × 1360. (A.I.P. acc. 126110; neg. 86331.)

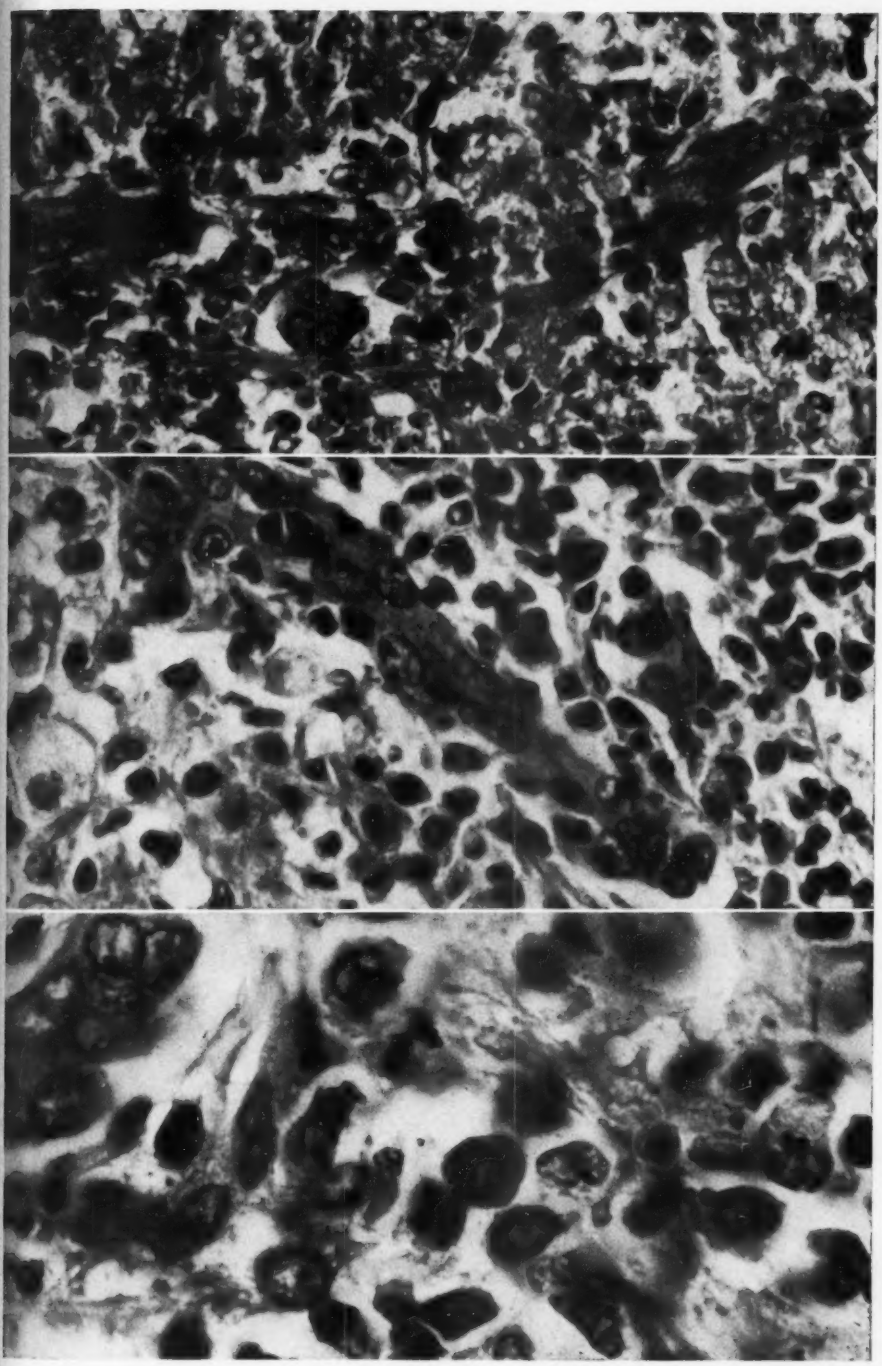


PLATE 173

FIG. 26. Duration of disease, 10 days. (See case 15.) Portal region of liver. Early proliferation of biliary ducts. The proliferating ducts have an irregular shape, due to budding and branching. The component cells are relatively large and have deeply chromatic nuclei. The stroma is densely infiltrated with inflammatory cells. × 230. (A.I.P. acc. 111844; neg. 86340.)

FIG. 27. Duration of disease, clinically less than 1 day. Spleen. The figure shows an enlarged follicle with a prominent and partly necrotic germinal center. (See also Figs. 8, 15, and 24.) × 300. (A.I.P. acc. 123465.)

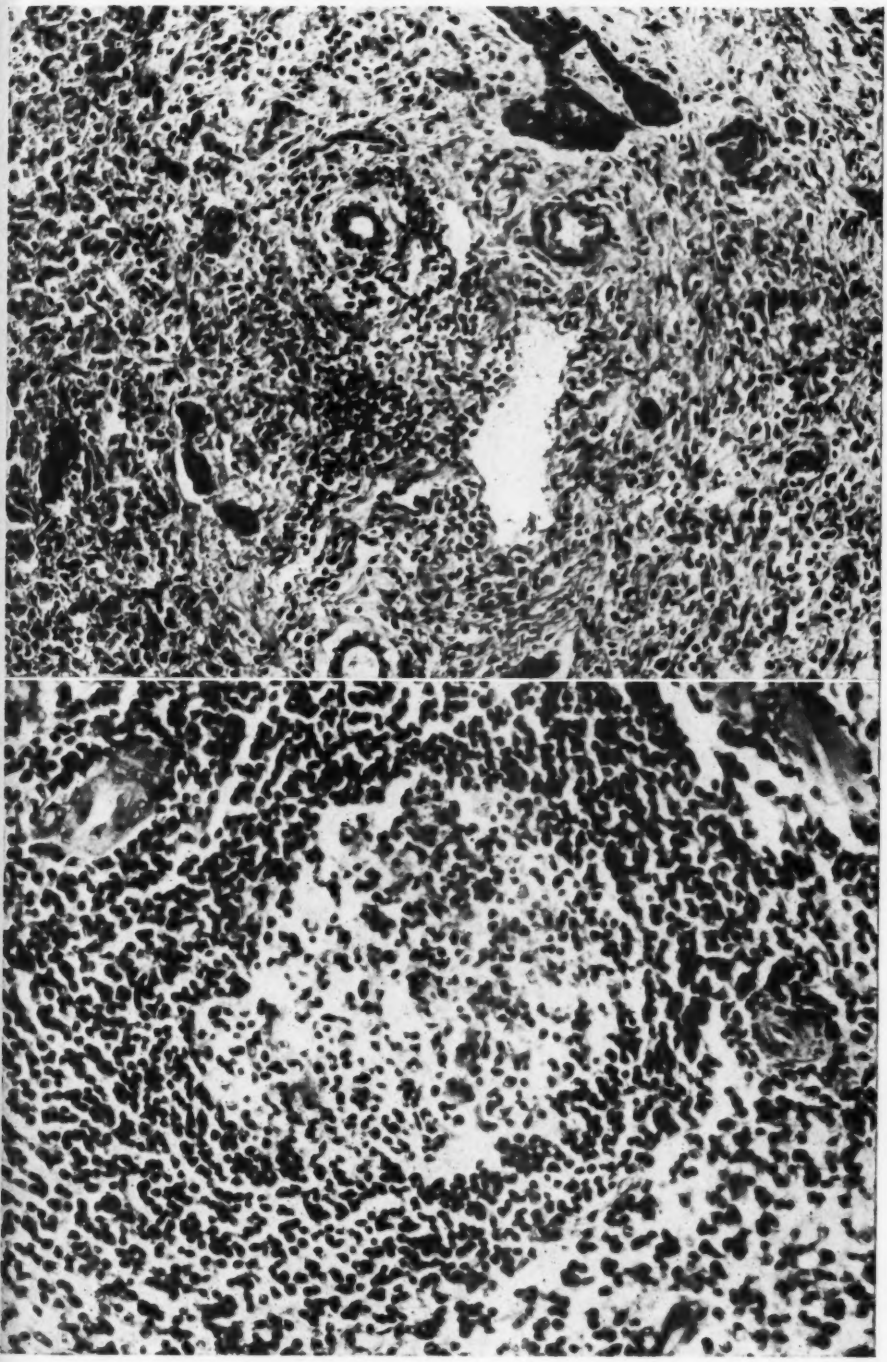


PLATE 174

FIG. 28. Duration of disease, 12 days. Appearance of liver at low magnification. The photograph shows a region in which destruction of hepatic parenchyma is incomplete. A thin, irregular rim of liver cells is preserved in the peripheral zone of the lobules. The portal regions and lobular remnants are densely infiltrated with inflammatory cells. (The section photographed was cut thick in order to bring out these features.) × 50. (A.I.P. acc. 123473; neg. 86017.)

FIG. 29. Another field from the liver shown in the preceding figure at the same magnification. In this area only occasional clumps of liver cells have been preserved. Where destruction is complete, the lobular remnants are outlined by proliferating bile ducts. The granular appearance of the interior of the lobules is the result of intense congestion and invasion by inflammatory cells. This and the preceding photograph give evidence that even in rapidly fatal cases of epidemic hepatitis involvement of the hepatic parenchyma is sometimes not uniform throughout the organ: while in large regions destruction may be complete, elsewhere patches of parenchyma are preserved. In the subacute form of epidemic hepatitis involvement of the liver characteristically is not uniform. × 50. (A.I.P. acc. 123473.)

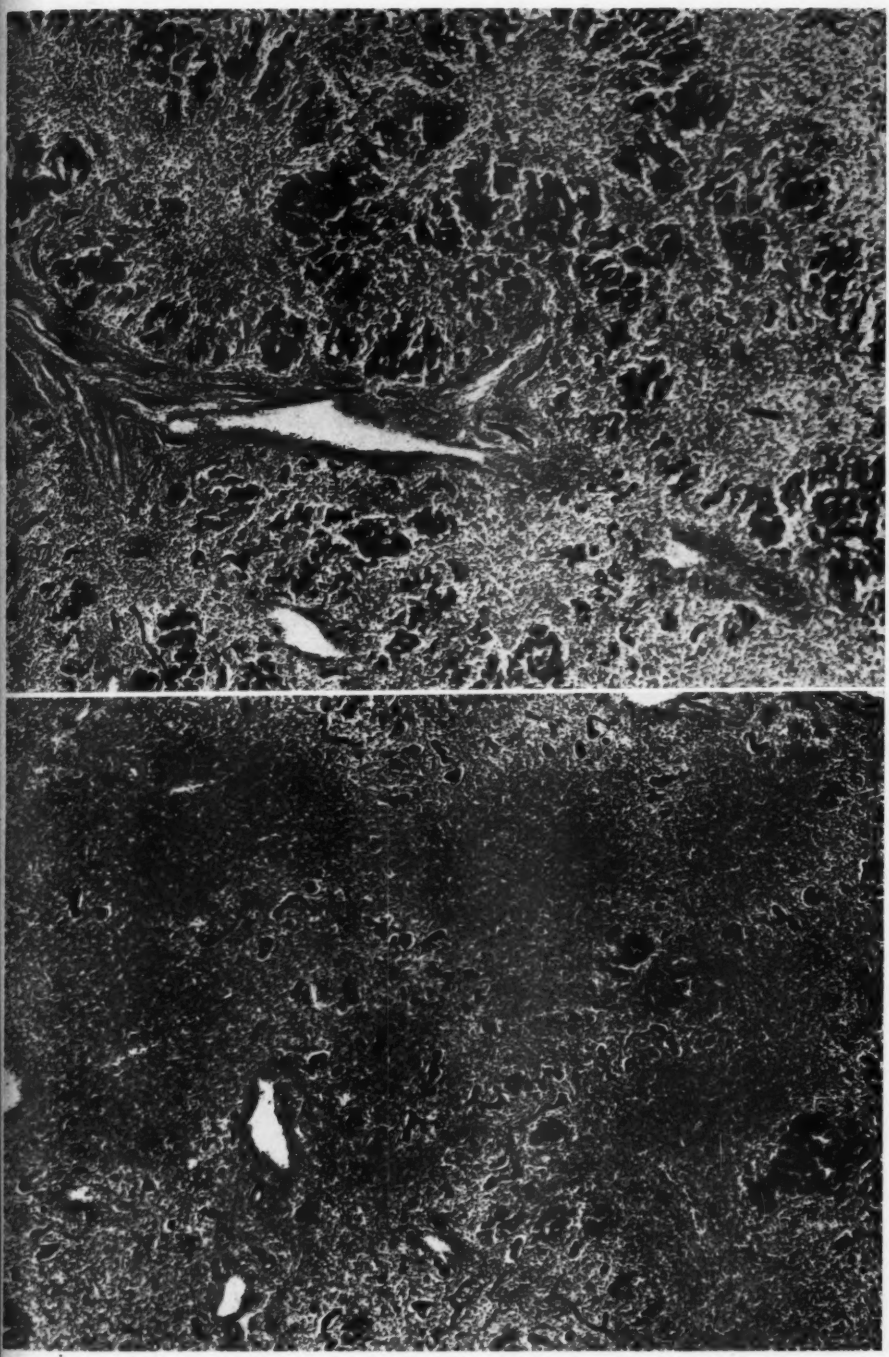
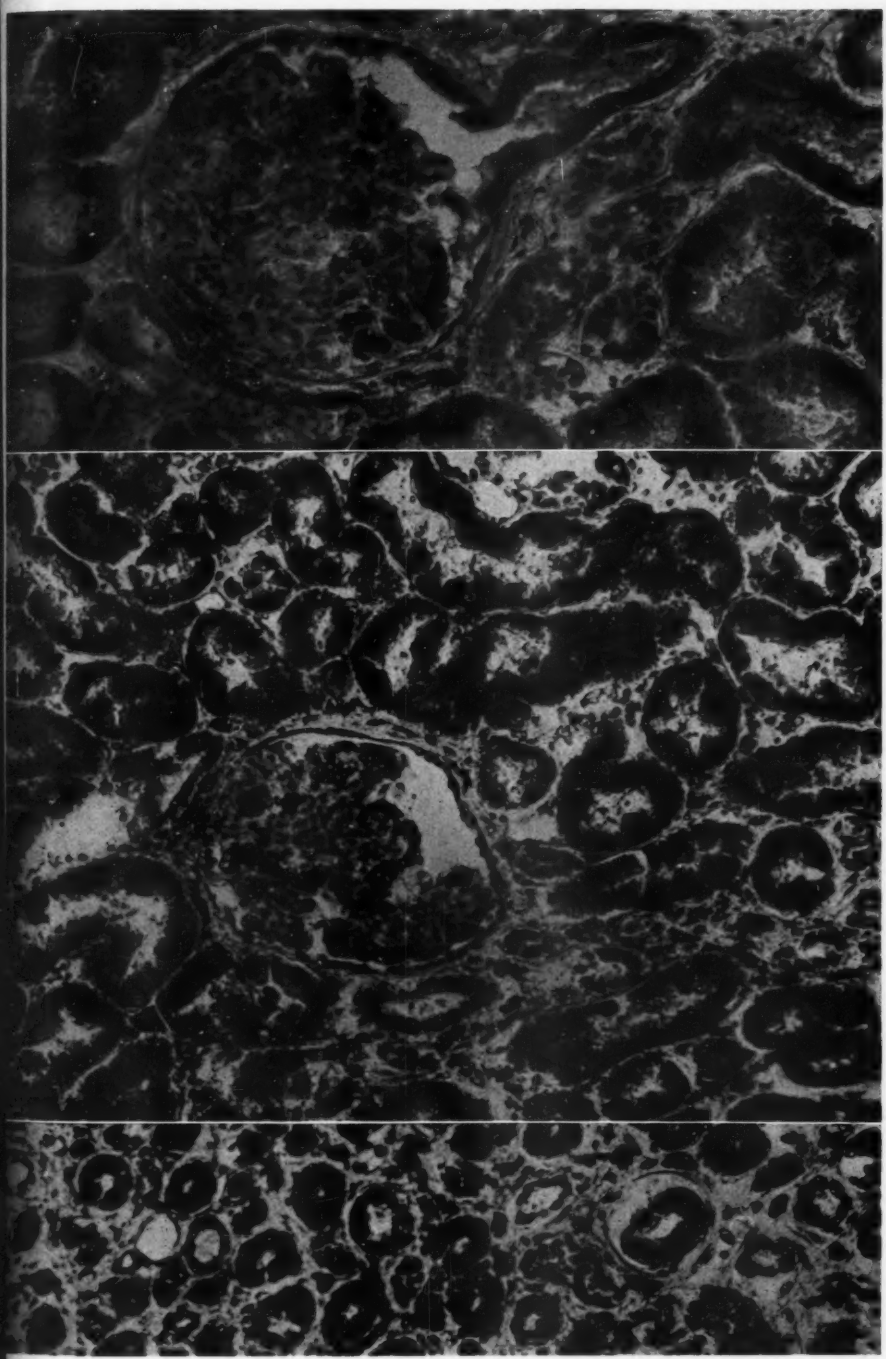


PLATE 175

FIG. 30. Duration of disease, 7 days. (See case report 9.) Kidney; thick frozen section, stained with sudan. The epithelium of the neck of the proximal tubule and the convoluted segments are packed with fat. In contrast, the distal tubules, adjacent to the glomeruli, contain very little stainable fat. × 230. (A.I.P. acc. 125602; neg. 87069.)

FIG. 31. Duration of disease, 4 days. (See case report 11.) Kidney; thick frozen section, stained with sudan. The cells of the proximal convoluted tubules are packed with fat which appears black in the photomicrograph. No fat is demonstrable in the glomerulus and very little in the adjacent loops of distal convoluted tubules. (See also Fig. 32.) × 175. (A.I.P. acc. 126811; neg. 87068.)

FIG. 32. Boundary zone of kidney from the same case as shown in Figure 31. Thick frozen section, sudan stain. The cells of the thick limbs of Henle are laden with fat, whereas those of the thin limbs and of the collecting tubules contain very little or no demonstrable fat. × 230. (A.I.P. acc. 126811.)



BONE INFARCTS

Case Report with Autopsy Findings *

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Infarction of soft parts and especially of certain internal organs is a generally recognized condition, but infarction of bones, whether in its early or late stages, is a condition that is little known to the general medical profession. One looks in vain for more than a bare mention of this subject in textbooks on general and special pathology such as those of Kaufmann, Aschoff, MacCallum, Karsner, and Boyd. Since the publication by Kahlstrom, Burton, and Phemister [1] of the pathological and roentgenological findings in a series of cases of old bone infarcts, which made it possible to establish the diagnosis roentgenologically, numerous reports of cases have appeared in the literature.

The roentgenological recognition of old infarcts is based on the fact that when they are located in the heads of the femora and humeri, use leads to collapse, followed by organization of the dead bone bordering on the joints and deforming arthritis; and when they are located in the shafts and deeper portions of epiphyses, they become partly replaced by new bone and partly calcified, and cast blotchy medullary and linear peripheral shadows that are denser than those of normal cancellous bone.

The reported lesions have been confined to the long bones and have been either single or multiple. They have occurred in workers in compressed air as a sequel of the picture of caisson disease and also in an equally large number of persons, both male and female, who have never worked in compressed air and in whom the cause is usually obscure. No report has been found of bone infarction produced by nitrogen liberation from too rapid ascent in an airplane. Additional cases occurring in caisson workers and presenting the typical x-ray findings have been reported by Coley and Moore,[2] Walker,[3] Rendich and Harrington,[4] Bell, Edson, and Hornick,[5] Taylor,[6] and others. Of the 54 cases in Taylor's report on infarction, 13 were due to caisson disease and the remainder had no occupational history. Bell, Edson, and Hornick studied 32 compressed air workers, of whom 14 gave a history of bends, 1 of otalgia, and 4 of arthritis. Twenty-four, or 75 per cent of the number, showed typical changes of old infarction in one or more bones on x-ray examination.

* Received for publication, July 20, 1945.

There is still a dearth of cases that have been studied pathologically. Tissue excised at operation confirmed the diagnosis in the case reported by Walker.[3] One of us (S. C. K.) has autopsied 3 cases in a 6-year period in a very limited service, which speaks for the relative frequency of this entity. The other [7] has had an opportunity to examine an infarct of the tibia obtained at autopsy and to confirm the diagnosis in case no. 4 of our original report (Part I) with Burton,[1] by examination of tissue since removed in an operation on the hip.

There are good reasons for the comparative silence on this subject in the literature of pathology. In the first place, many bone infarcts are entirely asymptomatic, especially for many weeks or months after their development and are therefore no cause for concern to the patient, the clinician, or the pathologist. Their discovery during life, as in the case here presented, is often entirely accidental. However, if they involve the end of the bone and lead to either collapse of the articular portion or to chronic arthritis, the pain and limitation of motion in the joints frequently lead to the establishment of the diagnosis by roentgenological examination. The striking absence or paucity of reports of infarction observed in either the early or late stages is related to the relatively infrequent examinations of the bones of the extremities at autopsy and to the infrequency with which operation gives an opportunity to obtain tissue for microscopical examination. There is every indication that if the large long bones of the body were routinely studied roentgenologically before autopsy and were sectioned longitudinally, there would be an enormous increase in the frequency of recognition of recent and old skeletal infarcts. The careful visceral necropsy usually suffices to explain the clinical manifestations and cause of death and this too often lessens the incentive to undertake the laborious task of removing the bones for further study. Roentgenograms are not taken because of the time and expense involved. The embalmer and the relatives often object to detailed skeletal examination because of mutilation of the body.

The more careful study, with sectioning of bones, of extremities amputated for arteriosclerotic gangrene would doubtless reveal evidence of blockage of blood vessels in the bones, now frequently overlooked. The departments of anatomy of medical schools have access to bones which could be routinely sectioned for gross examination, at least, without great trouble or expense. The material aid offered by x-ray examination of bone specimens in a study of this type would stimulate great interest in the correlation of the status of bone pathology with visceral pathology.

Report of Case

While the clinical and roentgenological characteristics of the following case have been reported previously,[8] the subsequent verification of the bone lesions by autopsy forms the basis for this paper.

J. K., a Polish male, 55 years of age, was hospitalized at U. S. Veterans Administration, Bath, N. Y., for the sixth and final time in September, 1942, for recurrent hemorrhages from the gastrointestinal tract. The diagnoses previously established were cirrhosis of the liver with esophageal varices, secondary anemia, diabetes mellitus, arteriosclerosis, coronary and general cardiac enlargement, bilateral varicose veins, and multiple old bone infarcts.

The family history was irrelevant. The patient's only occupation had been that of chef. There was no history of exposure to compressed air. He had at times been addicted to excessive use of alcohol.

The bone lesions were discovered quite by accident, the left knee being radiographed following a minor injury in 1940. No fracture or dislocation was present, but large, irregular, mottled calcific deposits of increased density were present in the medullary portions of the distal third of the left femur and the proximal third of the left tibia. Following roentgenographic survey of the remaining skeleton, similar lesions were encountered in the distal third of the right femur and the middle third of the left humerus, the latter being but a small fleck of increased density.

The patient was rather stocky and well developed, but weak and quite anemic on this admission. The pulse was 92, respirations were 28, and the blood pressure was 94/70 mm. Hg. The physical examination further revealed a slightly enlarged liver, but was not otherwise remarkable. The stools were strongly positive for occult blood. The red blood cells numbered 2,700,000 with a hemoglobin of 45 per cent; the urine contained 1 per cent sugar while the blood sugar was 250 mg. per cent. As on many previous occasions, no source for the hemorrhage could be found in the x-ray examination of the gastrointestinal tract. Esophageal varices were looked for, but not found. The stomach was filled with a large blood clot.

The patient was given supportive treatment, including repeated blood transfusions, but continued to have hemorrhage and expired on October 21, 1942.

Autopsy disclosed a ruptured esophageal varix; cirrhosis of the liver; ascites; amyloidosis of the spleen, with perisplenitis; arteriosclerosis, coronary and generalized; cardiac enlargement (400 gm.); chronic interstitial nephritis; and an accessory kidney on the right side. The left tibia and each femur were removed. They were studied in the Laboratory of Surgical Pathology of the University of Chicago.

The external appearance of all three bones was not remarkable; the articular cartilages and synovia appeared normal. They were sectioned midcoronally. The proximal half of each femur was filled with red bone marrow, whereas the distal half contained fatty marrow except in the regions of the lesions. In the right femur, beginning 7 cm. above the lower end and extending upward for 2 cm., was a jagged, yellowish white, hard, amorphous area surrounded in most of its extent by a narrow, brownish, peripheral zone (Fig. 1). In the posterior half of the left femur, beginning 10 cm. above the lower end and extending

upward for 9 cm., the medullary content was dark gray with a mottled whitish streak of hard chalky material in the upper half (Fig. 2). Penetration with a needle revealed hard deposits in the posterior half at this level.

On section the left tibia contained yellow marrow throughout except in the upper third of the shaft. In the latter region a mottled lesion was encountered which filled the medullary canal irregularly for a distance of 10 cm. (Fig. 6). It was yellowish to gray in its mid-central portion. There were two dark brown patches, a large one superomesially and a small one inferolaterally, and a narrow dark brown zone lined the internal surface of the cortex along most of the involved segment. Roentgenograms were made of coronal sections cut approximately 0.5 cm. thick from the involved regions of the femurs (Figs. 3 and 4) and left tibia (Fig. 7). They bring out well the details of the dense mottle shadows of the lesions in the medullary canals. The slices were then decalcified and microscopical sections were made large enough to include the entire extent of the lesions. They were stained with hematoxylin and eosin. Figure 5 shows the unenlarged microscopical section of the left femur. In the upper half the marrow cavity is occupied by irregular islands of pale eosin-staining material surrounded by zones of blue-staining tissue of varying degrees of thickness. In the central portion of the lower half is a similar oblong lesion surrounded by normal appearing bone marrow. Under magnification it is seen that the pale central regions are necrotic and unorganized, while the hematoxylin-staining surrounding areas represent necrotic tissue that has been invaded by connective tissue which has become calcified in varying degrees and at the periphery has in turn been replaced by new bone or marrow.

Figure 9 shows a low magnification of the region indicated by "×" in Figure 5. Zone *a* is the outer cortex consisting of living bone which, from its normal arrangement, appears not to have been involved. Zone *b* is the inner cortex which is irregularly arranged and is composed principally of living bone and, to a small extent, of dead bone which is undergoing creeping substitution by new bone. In this region there appears to have been extensive necrosis which, for the most part, has been repaired. Calcium granules have been deposited in some of the haversian canals. At *c* is the zone of fibrous invasion of the necrotic endosteal and medullary region, with marked calcification along the inner margin. The old trabeculae are dead and surrounded by connective tissue. In the peripheral regions some of the calcified connective tissue is undergoing ossification and some of the dead trabeculae are being replaced by new bone. Zone *d* is the central necrotic

medullary region. It is, for the most part, filled with amorphous débris throughout which calcium granules are irregularly dispersed. Scattered, small, necrotic, bony trabeculae are present. Throughout the region there are vacuoles with calcium more densely concentrated at the periphery. Regions from the zones of Figure 9 are shown in detail in Figures 10 to 12. Figure 10 is from zone *b*. It shows dead bone, A, undergoing creeping replacement by new bone, B. Figure 11, from zone *c*, shows the dead trabeculae remaining, but the dead marrow is replaced by fibrous tissue, a part of which has become calcified. Figure 12, from zone *d*, shows calcium granules in the necrotic débris about vacuoles, and partly calcified connective tissue invading the periphery. In some regions the outlines of old fat cells are to be seen, with calcifying débris filling the space formerly occupied by the cytoplasm. Cholesterol slits are present in a few regions, suggesting the previous existence of hemorrhage.

The elongated island in the center of the medullary cavity in the lower part of Figure 5 was similar to the lesion above it, to which it was attached in a posterior plane as revealed by the roentgenograms. There is evidence that in places it was still being very slowly reduced in size and replaced by fatty marrow and fine bony trabeculae about the periphery. Blood vessels were relatively sparse throughout the entire section and no old obstructed vessels were seen.

Sections of the smaller lesion in the right femur showed changes identical with those of the left femur.

Figure 8 shows a microscopical section of the tibial lesion which measured 8 cm. in length and filled almost the entire medullary canal for a length of 6 cm. Its upper and lower limits are jagged and there is a small, similarly involved island 1 cm. above the mass on the mesial side of the bone. There is a bluish-staining calcified zone about its periphery which in some places borders on the cortex of the shaft and in others is separated from the shaft by a narrow zone of marrow. The contents within its calcified zone are dark brown about the periphery and gray to brown in the central region. Microscopical examination shows them to be composed of necrotic fat, partly calcified and with the outlines of many of the cells preserved, old blood pigment especially abundant about the periphery, cholesterol slits, coarse calcium granules, necrotic bony trabeculae, and invading connective tissue. Figure 13 is a low-power view extending through cortex, calcified zone, old blood pigment containing cholesterol slits, and necrotic and partly calcified marrow. Figure 14 is a higher power showing old pigment containing cholesterol slits, necrotic and partly calcified fat, and necrotic bone trabeculae. The blood vessels of the bone outside the

infarct are small and there are no signs of obliteration or of arteriosclerosis.

Discussion

The three lesions which have been described are the remains of old aseptic infarcts which have been somewhat reduced in size by connective tissue invasion, absorption, and replacement by new bone and marrow at the periphery. The reparative stimulus appears to have been long since almost completely exhausted and the unreplaced portions have undergone partial calcification and encasement by a calcified fibrous wall. There was no appreciable change in the x-ray appearance between the first examination and that at death 2 years later. The presence of much blood pigment and of cholesterol slits in the tibial lesion, especially peripherally, is evidence that it was a hemorrhagic infarct. Cholesterol slits in a few regions of the left femoral lesion are suggestive of old hemorrhage there.

The location of the infarcts suggests that they arose from blockage of the branch of the nutrient artery supplying the involved end of the respective bone. The large size of the lesions in the left femur and tibia suggests that the entire branch may have been blocked, although the anteroposterior and lateral roentgenograms reveal that the posterior portion of the bone was the more extensively involved in each case. The small size and eccentric location of the lesion of the right femur would indicate blockage of only a portion of the inferior nutrient branch. The evidences of necrosis and creeping substitution of internal cortical bone in the left femur speak for nutrient artery blockage since it supplies the internal portion of the cortex.

The exact cause of this multiple infarction cannot be stated. There was no history of work under compressed air. The structure indicates that the lesions are all of the same vintage and that they are many years old. Because of the arteriosclerosis, both generalized and coronary, one must think of vascular blockage from intimal thickening, embolism or thrombosis. The absence of infarcts in the spleen and kidneys militates to some extent against that explanation. There was no evidence of old valvular lesions. The cirrhosis of the liver should be considered since Åxhausen [9] reported fresh infarcts in a patient dying with portal cirrhosis. The absence of recent infarcts speaks against cirrhosis as a causative factor.

Summary and Conclusions

Autopsy studies are reported of another case of ancient infarction in multiple bones of the extremities which was diagnosed roentgenologically before death by the presence of blotchy medullary shadows

of increased density produced by calcification of the unresolved portions of the infarcts.

The exact cause of the infarction was undetermined although it may have been related to the accompanying generalized and coronary arteriosclerosis.

A review of the recent literature indicates that old calcified bone infarcts, both multiple and single, are being diagnosed frequently by the blotchy shadows and evidences of collapse and deformity of articular surfaces shown in roentgenograms.

Failure to examine the long bones routinely at autopsy is responsible for nonrecognition of a great many infarcts.

Routine roentgenography of the bones of the extremities preceding autopsy would assist greatly in the recognition of old infarcts.

A diligent routine search for bone infarcts at post-mortem examination would lead to the discovery of lesions in the early stages and help to arrive at the cause in those cases which remain unexplained.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 176

FIG. 1. Yellowish white, calcified infarct in right femur.

FIG. 2 Grayish white, calcified areas in left femur.

FIG. 3. X-ray shadow of calcified infarct in right femur.

FIG. 4. X-ray shadow of calcified infarct of left femur.

FIG. 5. Microscopical section of calcified medullary infarcts of left femur.

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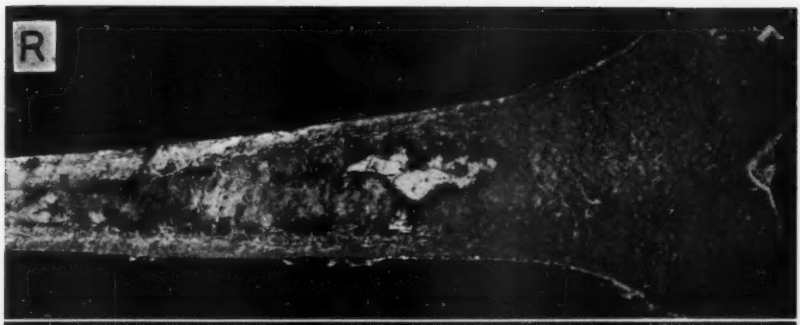

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Kahlstrom and Phemister

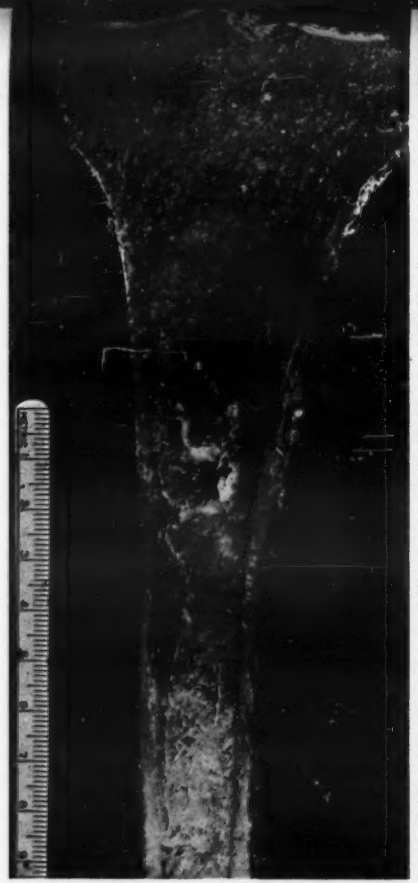
Bone Infarcts

PLATE 177

FIG. 6. Old hemorrhagic and calcified infarct of tibia.

FIG. 7. Roentgenogram of specimen illustrated in Figure 6, showing mottled increase in density of involved medullary region.

FIG. 8. Microscopical section of tibial lesion.



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Kahlstrom and Phemister

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8

Bone Infarcts

Plate 178

Fig. 9. The are marked "×" in Figure 5 at 8½ ×:

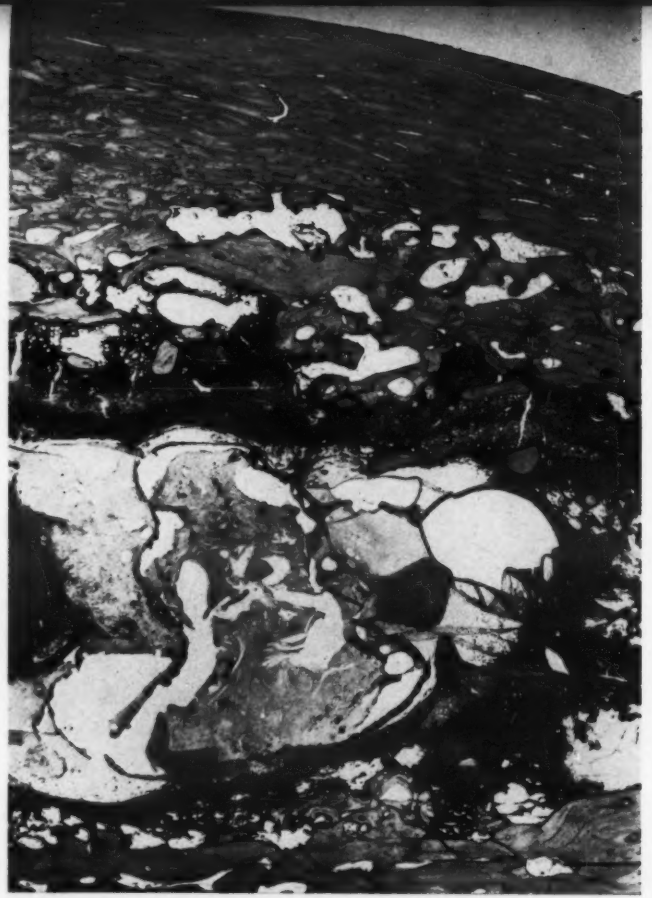
a. Unaltered superficial cortex.

b. Deep cortex, necrotic, and partially replaced by new bone.

c. Fibrous invasion and calcification of necrotic medullary region.

d. Liquefaction and calcification of medullary region.

Fig. 10. Zone *b*, Figure 9, at 190 ×, showing dead bone, A; being replaced by living bone, B.



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Bone Infarcts

PLATE 179

FIG. 11. Zone *c*, Figure 9, at 70 ×, showing fibrous replacement and calcification.

FIG. 12. Zone *d*, Figure 9, at 45 ×, showing necrosis, liquefaction, calcification, and connective tissue invasion.

11

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12

Bone Infarcts

PLATE 180

FIG. 13. Level "×" of Figure 8, at 18 ×, passing through cortex, *a*; calcified zone, *b*; old blood pigment and cholesterol slits, *c*; and necrotic and partly calcified marrow, *d*.

FIG. 14. The central region of Figure 8, at 50 ×, showing necrotic fat with regional calcification, *a*; and old hemorrhage and cholesterol slits, *b*.

a b c d

a b

THE PATHOLOGY OF JAPANESE B ENCEPHALITIS *

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During the second week of July, 1945, instances of an acute febrile nervous disorder began making their appearance on Okinawa-Jima in the Ryukyu group of islands. These first occurred among the native population, but sporadic cases of the disease began to appear in the U. S. military personnel stationed on the island. Increasing numbers of cases were reported during the second half of July, the level of incidence being maintained until the last week of August, when it gradually began to fall. An occasional case, only, was encountered in September. In all, there were about 30 instances of this condition in our military personnel and perhaps 200 to 300 in the natives.

Many factors contributed to the almost insurmountable difficulties encountered in determining the true incidence in the native Okinawans. In the first place, a large portion of the population was displaced from the lower to the middle third of the island, where the unavoidably crowded living conditions produced a sharp rise in the incidence of many confusing infectious diseases. Also, frequent shifting of large groups of the population from place to place necessitated the repeated parallel transfer of Military Government hospital facilities with consequent interruption of service. Then, the language barriers between the natives and the medical personnel of our Military Government all but prevented obtaining reliable clinical data. Whether because of stoicism, fear, or indifference, many of the cases among the natives, at least at first, were not reported, and, thus, it became necessary for some of our medical officers to devote most of their efforts to seeking out the natives stricken with the disease. This was time-consuming and inefficient, but undoubtedly served to locate many cases which otherwise would have gone unrecognized.

The failure to uncover some cases of this malady of the nervous system was counterbalanced by the inclusion of many instances of nervous diseases of other types. This was especially true after the U. S. Military Government Research Center E-1 was opened as the receiving hospital for such patients. On the roster of patients in this hospital were many with tuberculous meningitis, other meningitides, and malaria.

For these several reasons, accurate knowledge of the incidence of

* The Bureau of Medicine and Surgery of the U.S. Navy does not necessarily undertake to endorse the views expressed in this paper.

Received for publication, November 29, 1945.

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the disease and its mortality rate is lacking. In view of the fact that but roughly 200 natives were involved in a population estimated at about 300,000, the nervous disorder failed to reach epidemic proportions. Endemics such as this one, thought to be due to the virus of Japanese B encephalitis, were known by certain Japanese physicians to occur in the summer months on Okinawa. For this reason, study of the present endemic was begun immediately to determine its etiology, mode of transmission, and morphologic features. Various members of the U. S. Naval Medical Research Unit No. 2, as well as the staff of the Military Government Research Center E-1, participated. This report will deal with the anatomic findings in 11 fatal cases of the

TABLE I

Fatal Cases of Japanese B Encephalitis Listed According to Duration of Illness

Case no.	Clinical case no.	Sex	Age	Duration of illness
			(*years*)	(*days*)
1	—	M	*ca.* 20	5
2	—	M	*ca.* 20	6
3	10	F	6	6
4	12	M	2	6
5	18	F	7	7
6	13	M	7	8
7	5	M	8	11
8	9	F	21	13
9	31	F	5	37
10	72	F	6	48
11	36	F	14	52

disease which were proved with reasonable certainty to be due to the virus of Japanese B encephalitis.

Summary of Clinical Course. The patients in the fatal group presented a remarkably uniform clinical picture.* In each instance where this information is available (8 of the 11 cases), the onset of the illness was sudden with fever and headache. In addition, 3 of the patients became somnolent, lethargic, or drowsy and 2 had generalized convulsions. Prominent among the neurologic signs in the course of illness were nuchal rigidity, positive Kernig's sign, stupor, loss of speech, generalized convulsions, flaccid paralysis, Babinski's sign, and absent abdominal reflexes. Three of the patients had hyperreflexia, 2 had trismus, and at least 2 others had dissociated ocular movements. Three of the patients who survived more than 30 days gradually became spastic, were comatose, and lapsed into a peculiar "vegetative" state.

In Table I are listed the cases in chronologic sequence according to

*I am indebted to Lt. Comdr. Leon Lewis, M.C., U.S.N.R., Lt. Comdr. Lewis Thomas, M.C.(S), U.S.N.R., and Lt. John L. Peck, M.C.(S), U.S.N.R., for the clinical records of these cases.

the duration of illness. The first patient was a private in the U. S. Army and the second was a sergeant in the U. S. Marine Corps Reserve. These 2 represented the only fatal cases in military personnel. The remaining 9 patients were native Okinawans. All patients who died were under 25 years of age.

Complement-Fixation Tests. The presence of complement-fixation reactions with the virus of Japanese B encephalitis and the sera from cases 9, 10, and 11 was demonstrated by Lt. Comdr. Horace L. Hodes, M.C.(S), U.S.N.R. On the 5th day of illness, the serum from case 9 had no complement-fixing properties, but on the 19th day the complement-fixation test was positive (4 plus) in a dilution of 1:16 and weakly positive (1 plus) in a 1:32 dilution. Two days before death, however, the test again became negative. The serum from case 10 was weakly positive (1 plus) for complement-fixation in a dilution of 1:64 on the 9th day of the disease, but it was strongly positive (4 plus) in a dilution of 1:124 on the 31st day of illness. The complement-fixation test with the serum from case 11 was negative on the 3rd day of the disease, but on the 32nd day a positive reaction (3 plus) was obtained in a dilution of 1:32. These findings indicated that the nervous disorder to which the patients listed in Table I succumbed was caused by the virus of Japanese B encephalitis.

Anatomic Changes in the Nervous System

The nervous systems and other viscera of all but 4 of the fatal cases were removed at necropsy by several different pathologists, sectioned, placed in 10 per cent neutral formaldehyde, and sent by air to the Laboratory of Pathology of the U. S. Naval Medical Research Unit no. 2 on Guam. I returned from Okinawa with the material from the 4 remaining cases, and the preparation of microscopic slides of all the tissues was begun in our laboratory. Special attention was devoted to the nervous system, and a variety of different staining methods was employed for the demonstration of ganglion cells, glia, medullary sheaths, axis cylinders, and fat.

Macroscopic Findings

The nervous tissue and meninges of the patients who died in less than 2 weeks of illness disclosed but few findings on macroscopic examination. The meninges were not clouded by exudate and none contained an excess of cerebrospinal fluid. Congestion of the pial vessels was noted, but was not extreme. In several cases, small foci of congested blood vessels and clusters of petechiae were found in the cerebral cortical gray matter and in the basal ganglia. Such vascular

lesions, however, were neither extensive nor constant as to location. In two of the spinal cords, the central gray matter of the cervical region was discolored as in acute poliomyelitis. On the whole, however, the changes found in the acute cases on naked-eye examination were unimpressive.

In the 3 patients who survived more than a month, cerebral lesions were easily observed and were of rather unusual types. Whereas, again, the pia-arachnoid and the meningeal vessels were not remarkable, the cortical gray matter of both cerebral hemispheres was altered in a characteristic fashion. Patches of different size, sometimes pale because they were apparently acellular and at other times finely granular and distinctly gritty, were found in the cortical gray matter of every lobe and nearly every gyrus. A sensation as if cutting through calcified tissue was produced when the brains were sectioned. These lesions had no predilection for either the troughs or the crests of the gyri. Similar but larger lesions were found in the central gray matter, where the pallidal nuclei, the thalami, the red nuclei, and the substantiae nigrae appeared to be involved selectively. In addition, cystic changes resembling encephalomalacia were seen both in the pallidal nuclei and the substantiae nigrae. In the pontile and dentate nuclei of the cerebellum there were faintly visible, minute foci of brown discoloration. Close examination of the cerebellar folia disclosed a peculiar blurring of the cortical pattern which was seen to a lesser extent also in one or two of the more acute cases. The diameters of the spinal cord of case 10 appeared to be decreased, and it was only with great difficulty that the outlines of the central gray matter could be visualized. This, again, was reminiscent of the changes in poliomyelitis.

Microscopic Findings

Microscopic examination of the nervous systems of these patients revealed a great variety of lesions. In the more acute cases the leptomeninges were infiltrated with sparse lymphocytes, which were somewhat more numerous in the depths of the cerebral sulci (Fig. 1). A few large mononuclear cells and macrophages were also found in the pia-arachnoid of the older cases, but the cellular exudate was never a very conspicuous feature. Also, as noted in the macroscopic description of the acute cases, foci of capillary congestion and "ring" hemorrhages were present in the basal ganglia (Fig. 2). Perivascular "cuffing" by lymphocytes and, on occasion, by monocytes and macrophages (some containing fat) was present in many different parts of the brain, including the white matter, where other types of pathologic change were absent (Fig. 3). Small round cells were found not only in the

Virchow-Robin spaces but also in the vascular sheaths. The perivascular inflammatory process was most evident in the spinal cord.

Far more noteworthy than the exudative reaction was the widespread neuronal destruction that occurred in the cerebral cortex, the basal ganglia, the pons, medulla, cerebellum, and spinal cord. The degree of neuronal involvement varied from case to case and from zone to zone in each case. The essentially degenerative changes in the ganglion cells were sometimes focal in extent, limited to small groups of these cells, as seen in the cerebral cortex illustrated in Figure 4, and sometimes involved all the cells of a specialized region, as in the cornu Ammonis formation (Fig. 5). The injured cells were often only 2 or 3 in a group, or as many as 20 or 30. Various types of cellular disintegration were noted. There were simple chromatolytic changes as well as the "severe" cellular change of Nissl. Many involved ganglion cells were shrunken and pyknotic, and some were eosinophilic. A few had pericellular incrustations, the pericellular spaces around others were widened, and some had become completely autolyzed and had disappeared. Where this involved larger groups of neurons, the affected areas had a characteristic spongy appearance.

The neuronal lesions were at times accompanied by the appearance of infiltrations of neutrophilic leukocytes as well as by proliferations of microglia (Figs. 6 and 8). Where the leukocytic response was excessive, the focal lesion assumed the characteristics of a miliary abscess, although rarely was the associated tissue necrosis of such extent as to suggest much suppuration. In the anterior horns of the spinal cord the glial and leukocytic reactions duplicated the histologic picture of acute poliomyelitis (Fig. 8). The injured anterior horn cells were invaded by both leukocytes and microglia in the process of neuronophagia (Fig. 7). The latter was rarely seen in regions other than the cord; elsewhere the damaged ganglion cells were indicated by increased glial satellitosis. Glial nodules as well as infiltrating lymphocytes, mononuclear cells, and polymorphonuclear leukocytes, similar to the reaction noted in the cord, were found in the caudate nuclei, the pallida, the red nuclei, the inferior olives, the cranial nerve nuclei in the floor of the medulla and, especially, in the substantia nigra (Fig. 9). As the melanin-containing ganglion cells of the latter region disintegrated, dark brown pigment granules were found scattered in the interstitial tissue. Just as in the cerebral cortex, when large numbers of neurons disappeared from the substantia, a spongy condition developed in this structure (Fig. 10).

The older lesions in both the cortical and central gray matter of the cerebrum developed a more complex histologic picture by the addition

of numerous macrophages (Figs. 11, 12, and 13). They still remained rather circumscribed, and the presence of some leukocytes coupled with necrosis of the interstitium conferred on them simultaneously certain features of encephalomalacia and also of abscess formation. The mononuclear phagocytes contained but sparse amounts of lipoid material. Most frequently these lesions were unrelated to blood vessels, but occasionally such a relationship did exist even if it appeared to be fortuitous (Fig. 12). In none of the cases were cerebral vascular occlusions found, although in one (case 11), a patient who developed a terminal necrotizing pneumonia, many cerebral vessels contained "leukocytic thrombi." It is emphasized here that these lesions were confined to the gray matter and were never seen in the white matter of any part of the nervous system.

There was still another type of commonly encountered lesion, the most characteristic features of which were its widespread dissemination, its circumscribed, focal appearance, and its almost complete lack of accompanying cellular fixed-tissue and exudative response (Figs. 14 to 20). Were it not for the fact that these changes were also limited to collections of ganglion cells, they could easily be mistaken for the lesions of multiple sclerosis. They involved the white matter only by extension from the gray, as occurred occasionally in the pons (Figs. 16 and 19). Even here, however, they were often quite sharply limited to the pontile nuclei, leaving the intervening medullary tracts unaffected (Fig. 20). Also, as sometimes occurred, when the lesion occupied a position in the sixth layer of the cerebral cortex, the subjacent white matter was spared (Fig. 17). Myelin sheath stains of such foci of injury disclosed an almost complete absence of fibers, with small local accumulations of fatty droplets. In sudan III preparations, more fat was demonstrated within phagocytes in the perivascular sheaths of the regional blood vessels. Ballooning, ribbon formation, and fragmentation of dendrites and axis cylinders were demonstrable with the Trelles * and Bielschowsky methods of staining (Fig. 18). Essentially none of these lesions was around or related to blood vessels. In many of the lesions the injured, disintegrating ganglion cells were still visible (Figs. 19 and 20). In some a feeble response on the part of the microglia and small round cells was in evidence (Figs. 16 and 17). In others, the disappearance of ganglion cells and interstitial glia left a barren field of delicate but disrupted fibrils. The fate of such lesions could be seen in the patient who survived 52 days (case 11), in whom their contraction by gliogenous scarring was evident in the injured pontile nuclei (Fig. 21).

* Trelles, J. O. Technique d'imprégnation argentique pour les coupes à la celloïdine. *Rev. neurol.*, 1932, 1, 459–460.

The peculiar cellular architecture of the cerebellar cortex in part accounted for the type of lesion seen in this structure. As elsewhere in the nervous system, the primary injury was to the ganglion cells, the Purkinje cells being more involved than the small granular cells. Along the dendritic processes of the former cell type, in the molecular layer, glial "lawns" appeared (Figs. 22 and 23). These were the invariable indication that the Purkinje cells were severely damaged, and they were present as often in the depths as in the crests of the cerebellar folia. They were the counterpart of the lesions seen in the cerebral cortex and illustrated in Figure 11. The second type of lesion encountered, namely, the spongy transformation of the Purkinje and molecular layers (Fig. 27), also had its complement in the cerebral cortex, basal ganglia, and pons (Figs. 14 to 20). These spongy areas were practically acellular and contained a fine fibrillary structure which in part was composed of degenerated dendrites (Fig. 24).

As noted macroscopically, the 3 patients in this series who survived longest had widely distributed deposits of calcium in the cerebral cortex and basal ganglia. In some regions, the particles of calcium salts were bunched in small patches (Fig. 25), while in others, such as the cerebral cortex, they replaced all the laminae in long, wide bands (Fig. 28). Von Kossa's silver nitrate method of staining proved that the essential nature of the fine, gritty particles in the tissue was calcium salts (Fig. 26). The coarser calcareous granules were found at sites where the preceding parenchymal injury was extensive enough to have resulted in large zones of encephalomalacia or in cystic degeneration (Figs. 29, 30, and 31). Evidently with time, the deposition of inorganic salts was added to and formed increasingly larger accumulations (Figs. 32 and 33). The walls of venules and arterioles became calcified, as was noted in children of 5, 6, and 14 years, respectively. In the wake of the deposition of calcium salts, an active foreign body giant cell response appeared. Particles of calcium salts were very often found engulfed by the multinucleated cells (Figs. 34 and 35). Also, numerous microglia, transformed into large mononuclear phagocytes, contributed their effort towards the removal of this material.

The ultimate outcome of certain of the lesions in the nervous system was either demonstrated in the more chronic cases or could be surmised. Mention has already been made of the reparative process initiated by the glia, chiefly fibrillary astrocytes, at the sites of focal encephalomalacia and cystic degeneration. The larger degenerated zones, after the débris was removed by phagocytosis, remained as cysts quite similar to those so often resulting from cerebral hemorrhage or infarction. It seems doubtful that much of the calcium salts which were deposited in the chronic lesions could ultimately be removed by phagocytosis.

Rather, it would seem that in nonfatal cases such lesions would, in time, be encapsulated either by fibrosis or fibrillary gliosis, or by both processes acting together towards the same accomplishment. What change followed the destruction of individual ganglion cells, which occurred throughout the brain but was best typified by the Purkinje cells, is illustrated in Figure 36. Here, a cerebellar folium is shown in which not a single Purkinje cell remained. This was characteristic of many of the cerebellar folia in all three chronic cases. It was noted that nothing remained of the glial "lawns" seen in the molecular layer of the acute cases. The Bergmann layer of glia showed no attempt at replacement gliosis, which often masks the loss of Purkinje cells. There was, also, a general diminution in the number of neurocytes in the granular layer of the cerebellar cortex.

Anatomic Changes in Other Organs

Organs other than the nervous system were available for detailed microscopic study from 3 of the acute cases (nos. 3, 5, and 7) and 2 of the chronic cases (nos. 9 and 11). Each of the 3 acute cases had typical bronchopneumonia of a noninterstitial type which was not suggestive of a virus etiology. This was the only lesion found; the other viscera were normal.

The patient who lived for 37 days after the onset of encephalitis (case 9) had an organizing pneumonia suggestive of a pneumococcal process. She, too, failed to show those changes which are associated with virus pneumonia. Her other organs contained no anatomic changes. The last patient (case 11) had a necrotizing pneumonia due to aspiration but, in addition, there were old caseous tuberculous lesions in parts of both adrenals. Ulceration of the gastric mucosa with underlying caseation and Langhans' giant cell formation were also present. Acid-fast organisms were demonstrated in both the adrenals and gastric ulcers. Tuberculous changes were not found in any other viscera, including the mediastinal and mesenteric lymph nodes.

Discussion

The histopathologic changes in 11 cases of widespread nonsuppurative encephalomyelitis were unlike those of the post-vaccinal and the post-infectious encephalitides. In the latter, the lesions are confined almost exclusively to the central white matter of the brain and the fiber tracts of the spinal cord. They are almost always perivascular in distribution and consist essentially of a breakdown of the medullary sheaths, accompanied by monocytic inflammatory changes. In the present cases, the white matter was spared except in those rare in-

stances where the lesions spread from affected collections of ganglion cells to encroach on the myelinated fibers. But primarily, the disease was one of injury to ganglion cells with secondary inflammatory, and regressive glial, changes. Certain of its more distinctive features, such as the acellular plaques in the cerebral cortex and basal ganglia, the "spongy" lesions in the cerebellar cortex, and the deposition of calcium salts in the chronic cases, conferred an individuality upon it, but it still resembled other forms of encephalitis of virus etiology. These are encephalitis lethargica (von Economo's disease), St. Louis encephalitis, and the American equine encephalomyelitides. It also has similarities to the now presumably extinct Australian-X disease and the common disease of sheep in Scotland known as louping ill.

A number of workers have previously described the pathology of Japanese B encephalitis. All have called attention to the perivascular cuffing with lymphocytes and the focal infiltrations of mononuclear cells in the meninges. Degenerative changes were noted in the nerve cells of the basal nuclei, substantia nigra, pons, and medulla, and small foci of necrosis were described in these structures. Bertrand and Miyashita [1] described definite plaques of demyelination in some of their cases. Presumably, these lesions were like those portrayed in the present communication, confined to the cerebral and cerebellar cortical gray matter and the basal ganglia, and were not like the lesions of post-infectious encephalitis previously mentioned, or of multiple sclerosis. Diffuse polymorphonuclear leukocytic infiltration was noted by Kingo [2,3] in the cerebrum, basal ganglia, and spinal cord. Kaneko and Aoki [4] drew attention to the formation of abscesses and widespread destruction of ganglion cells in this form of encephalitis. Other valuable contributions to the pathology of this disease have been made by Flexner,[5] Hashimoto, Kudo, and Uraguchi,[6] and Pette.[7] No one, however, has called particular attention to the outstanding destruction of Purkinje cells, the formation of glial "lawns" and "spongy" changes in the molecular layer of the cerebellar cortex, and the widespread deposition of calcium salts with its attendant foreign body response in the chronic cases.

It is fortunate that uncontroverted evidence is at hand to prove that the nonsuppurative encephalomyelitis described in this report is due to the virus of Japanese B encephalitis. This evidence, in the form of complement-fixation tests, was supplied by Hodes, Thomas, and Peck.[8]

Summary

The histopathologic findings in 11 cases of Japanese B encephalitis are presented; they may be summarized as follows:

There was focal as well as widespread destruction of ganglion cells in the cerebral cortex, basal ganglia, substantia nigra, red nuclei, cranial nerve nuclei in the floor of the fourth ventricle, inferior olives, cerebellar cortex, dentate nuclei, and cornua of the spinal cord. These destructive changes sometimes occurred alone, but were often accompanied by lymphocytic and polymorphonuclear leukocytic infiltration and by microglial proliferation. When these cellular reactions were associated with interstitial necrosis and phagocytosis by macrophages, focal lesions resulted which, on the one hand, resembled miliary abscesses and, on the other, patches of encephalomalacia.

In the cerebral cortex the injured ganglion cells were surrounded by increased numbers of glial satellites; in the cornua of the spinal cord there was neuronophagia.

The cerebrospinal meninges were usually infiltrated with small numbers of lymphocytes and monocytes. Perivascular collars of lymphocytes were present in the nervous parenchyma.

A few of the acute cases had capillary congestion and focal "ring" hemorrhages in the basal ganglia.

"Lawns" of glia appeared in the molecular layer of the cerebellar cortex at sites of Purkinje cell injury.

Acellular plaques were seen in the cerebral cortex, basal ganglia, and cerebellar folia. In these, there was destruction of the medullary fibers, dendrites, and axones. They had a spongy appearance and resembled the plaques of multiple sclerosis, except that they were confined to gray matter.

A reparative astrocytic gliosis occurred at the sites of smaller destructive lesions in the chronic cases.

The larger zones of encephalomalacia persisted as cyst-like lesions.

Focal and diffuse deposition of calcium salts took place at sites of injury in the chronic cases. These deposits stimulated a foreign body response with multinucleated giant cell formation.

The other viscera failed to show uniform or characteristic pathologic changes.

Acknowledgment is made of the valuable assistance of Robert H. Jackson, Pharmacists' Mate, 2nd Class, U.S.N.R., in histologic technic, and of Roy W. Poe, Pharmacists' Mate, 1st Class, U.S.N.R., in photography.

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8. Hodes, H. L., Thomas, L., and Peck, J. L. Cause of an outbreak of encephalitis established by means of complement-fixation tests. *Proc. Soc. Exper. Biol. & Med.*, 1945, **60**, 220–225.

[*Illustrations follow*]

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DESCRIPTION OF PLATES

All photomicrographs were made from preparations stained with hematoxylin and eosin, unless otherwise indicated.

PLATE 181

FIG. 1. Case 7. Lymphocytic infiltration in cerebral meninges. Membrana limitans not invaded. Mild astrocytic proliferation in molecular layer. × 100.

FIG. 2. Case 4. Capillary congestion and "ring" hemorrhages in basal ganglia. × 100.

FIG. 3. Case 7. Perivascular "cuffing" by lymphocytes in anterior horn of spinal cord. × 350.

FIG. 4. Case 5. Focal degenerations of ganglion cells in cerebral cortex. × 100.

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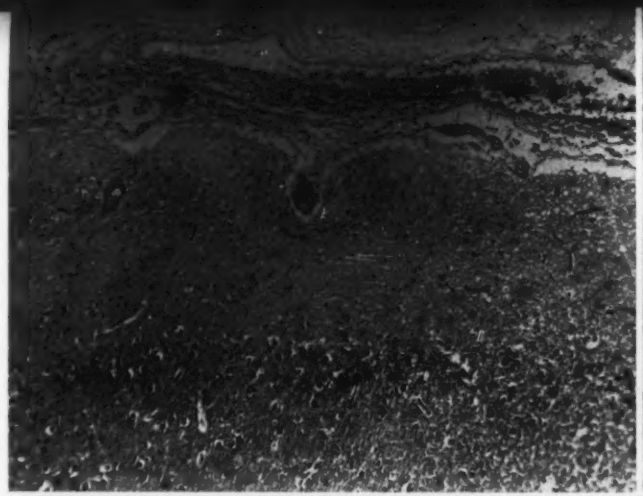

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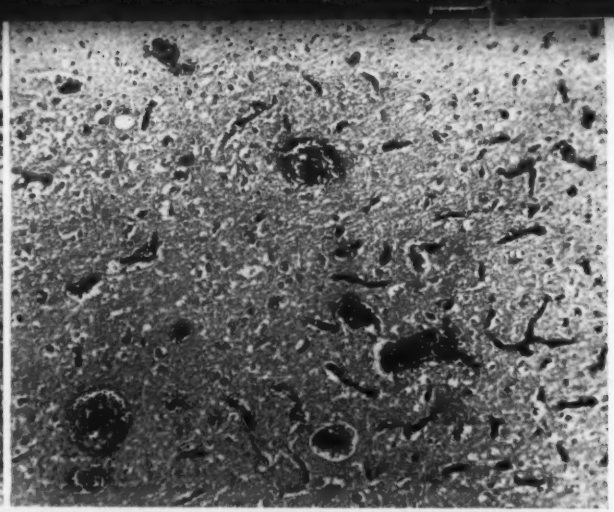

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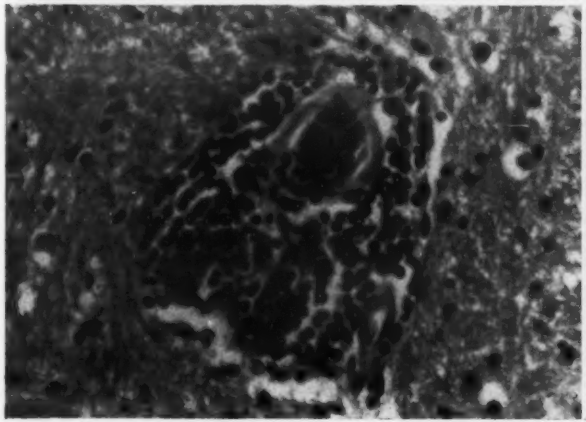

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Zimmerman

Japanese B Encephalitis

PLATE 182

FIG. 5. Case 3. Degenerative changes in all ganglion cells of Sommer's sector of the cornu Ammonis formation. × 100.

FIG. 6. Case 2. Focus of neuronal disintegration in cerebral cortex, with lymphocytic, polymorphonuclear leukocytic, and microglial response. × 350.

FIG. 7. Case 2. Neuronophagia of anterior horn cell of spinal cord. × 350.

FIG. 8. Case 2. Glial and lymphocytic reaction in anterior horn of spinal cord. Of note is the similarity to lesions in acute anterior poliomyelitis. × 100.

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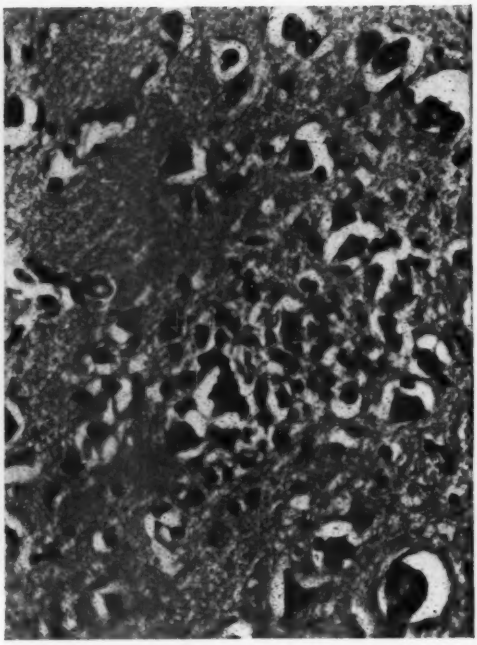

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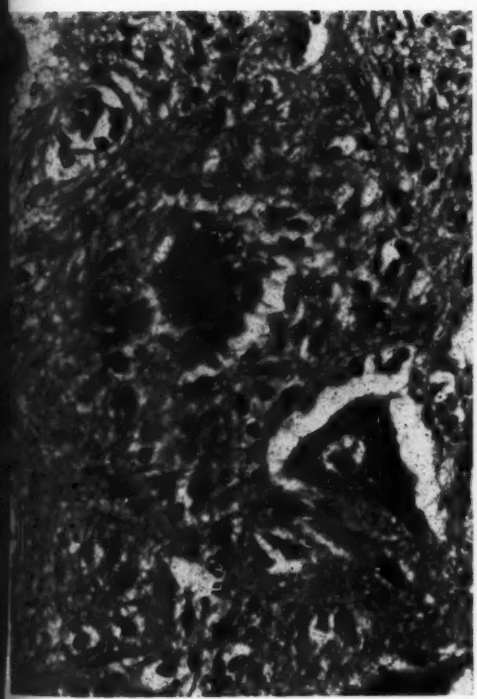

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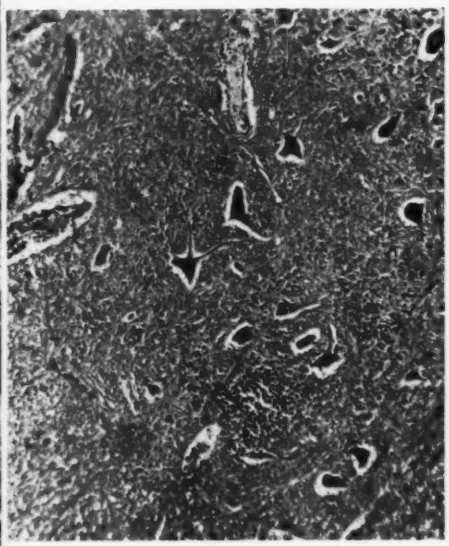

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PLATE 183

FIG. 9. Case 2. Nonsuppurative inflammatory reaction in substantia nigra. × 100.

FIG. 10. Case 2. Disappearance of melanin-containing neurons and "spongy" formation in substantia nigra. × 100.

FIG. 11. Case 5. Miliary abscess-like lesion in cerebral cortex. Macrophages, leukocytes, and interstitial necrosis. No relationship to blood vessels. × 100.

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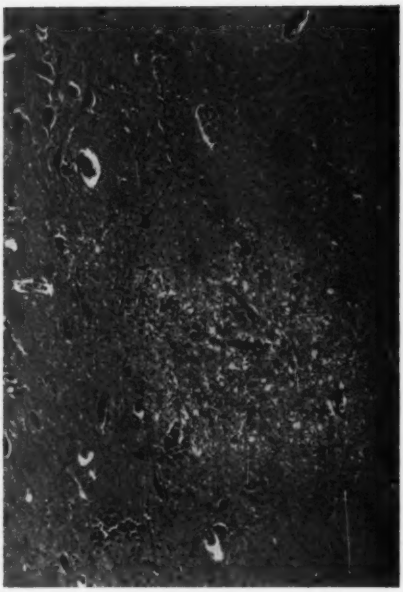

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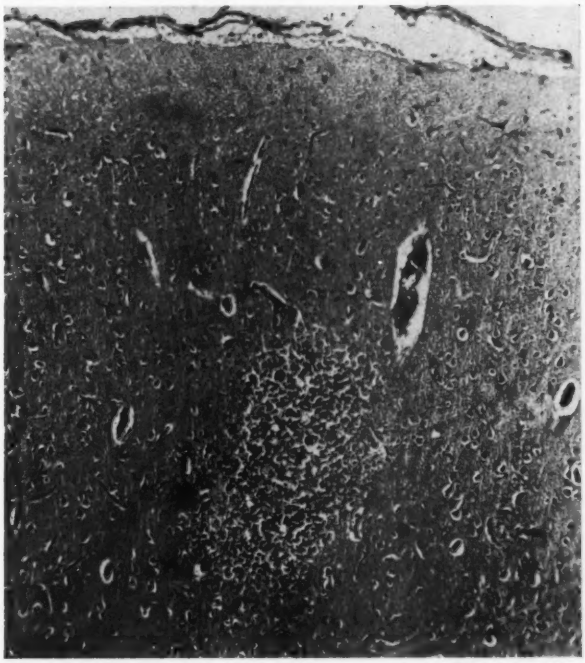

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PLATE 184

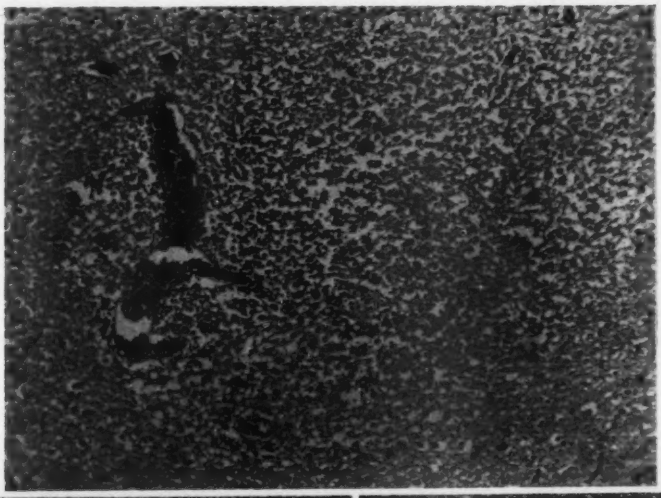
FIG. 12. Case 8. Lesion in basal ganglia similar to that illustrated in Figure 11, but related to venule. × 100.

FIG. 13. Case 4. Focal, nonsuppurative reaction in red nucleus. × 100.

FIG. 14. Case 5. Focal, acellular lesion in cerebral cortical gray matter. Superficially, like plaque in multiple sclerosis. × 100.

FIG. 15. Case 5. Multiple plaques in cerebral cortex. × 100.

FIG. 16. Case 5. Plaque in pontile nucleus containing a few small round cells. × 100.



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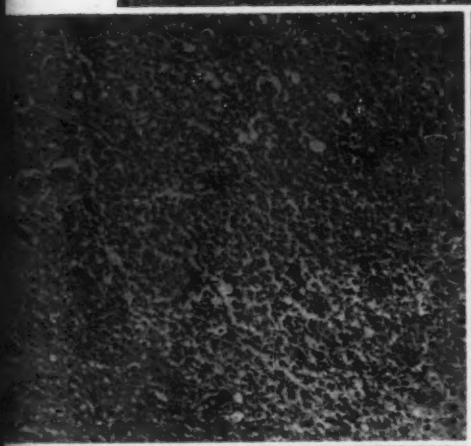

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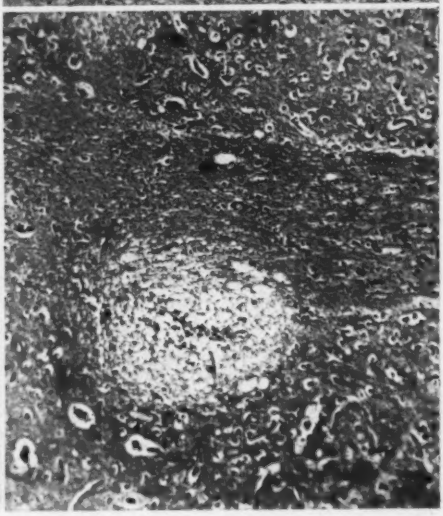

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Zimmerman Japanese B Encephalitis

PLATE 185

FIG. 17. Case 5. Focal lesion in sixth layer of cerebral cortex. No encroachment on subcortical white matter. × 100.

FIG. 18. Case 5. Fragmentation of axones and dendrites in cortical plaque. Trelles' stain. × 100.

FIG. 19. Case 6. Lesions in pontile nuclei encroaching on intervening fiber tracts. Ganglion cells in various stages of disintegration. × 100.

FIG. 20. Case 6. Lesion in pons sharply limited to nuclear mass. Adjacent white matter intact. × 100.

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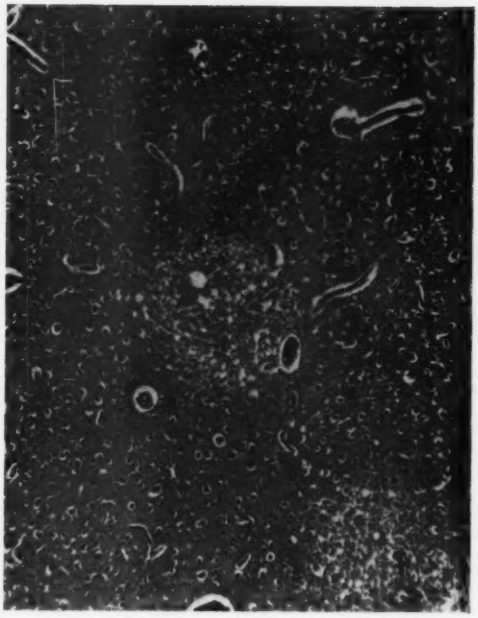

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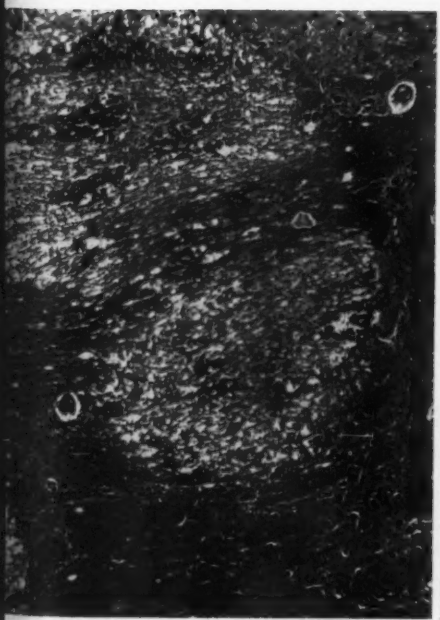

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PLATE 186

FIG. 21. Case 11. Sites of old injuries in pontile nuclei. There is contraction of the original lesions by scar tissue of gliogenous origin. × 100.

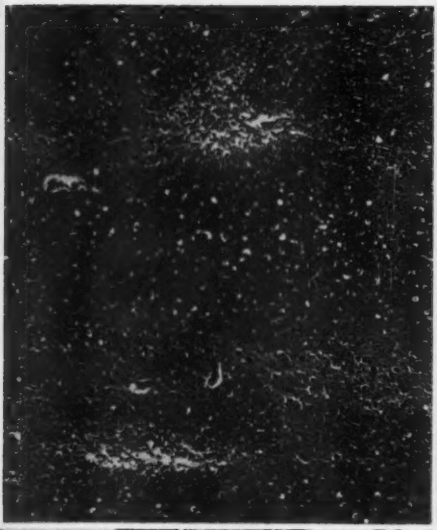
FIG. 22. Case 7. Glial "lawns" in molecular layer of cerebellar cortex at sites of injured Purkinje cells. × 100.

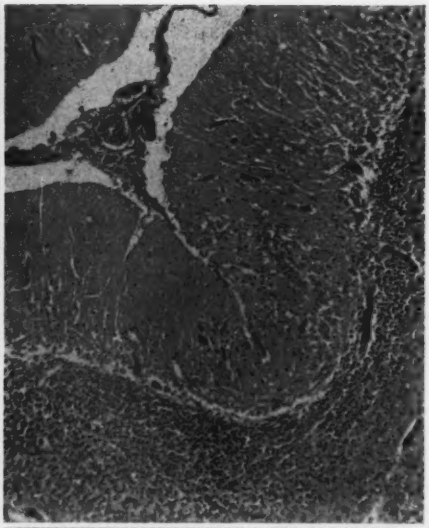
FIG. 23. Case 2. Purkinje cells absent and their former positions indicated by proliferation of glia in the molecular layer of cerebellar folium. × 100.

FIG. 24. Case 5. "Spongy" lesion in cerebellar cortex showing fragmentation and disappearance of dendritic processes. Trelles' stain. × 100.

FIG. 25. Case 11. Deposit of particles of calcium salts in thalamus. × 100.

FIG. 26. Case 11. Diffuse deposition of fine, black particles of calcium salts in cerebral cortex. Von Kossa's stain. × 100.





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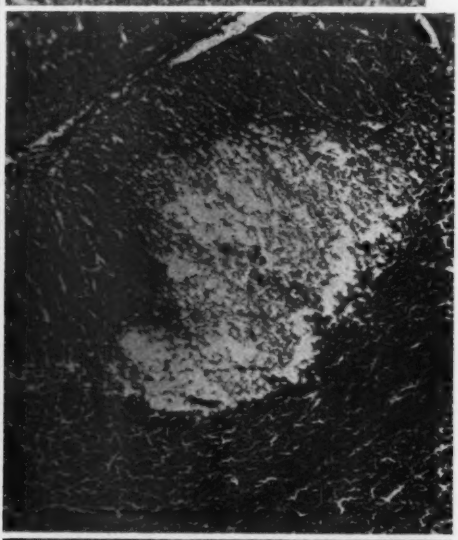

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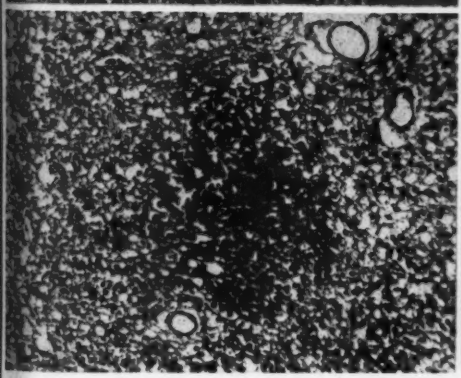

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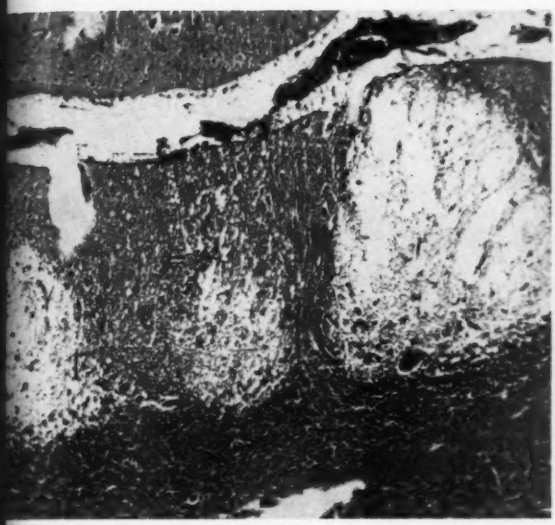
PLATE 187

FIG. 27. Case 5. Multiple plaques of degeneration affecting Purkinje cells and molecular layer. × 100.

FIG. 28. Case 11. Replacement of all the cortical laminae in the hippocampal gyrus by particles of calcium salts. × 100.

FIG. 29. Case 11. Patches of encephalomalacia with deposition of calcium salts in cerebral cortex. × 100.

FIG. 30. Case 11. Cystic degeneration in substantia nigra. Black particles are calcium salts. × 100.



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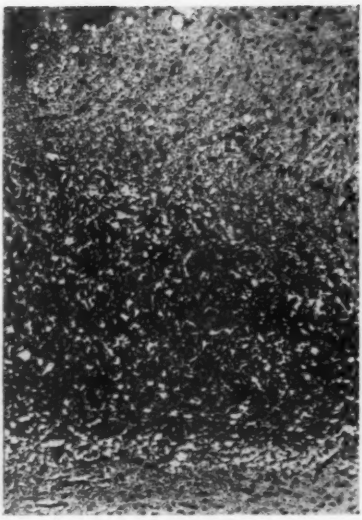

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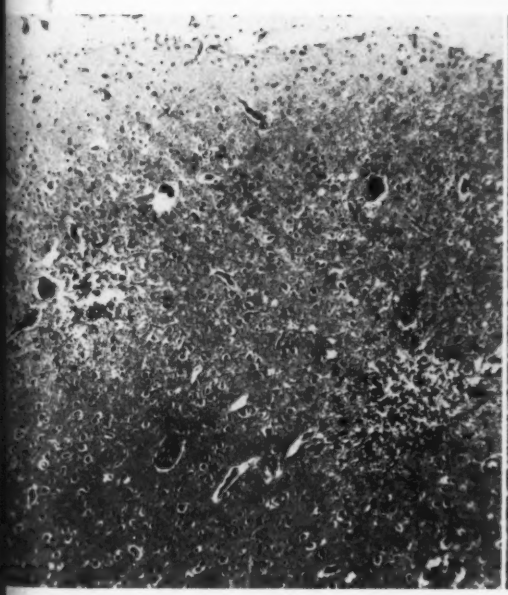

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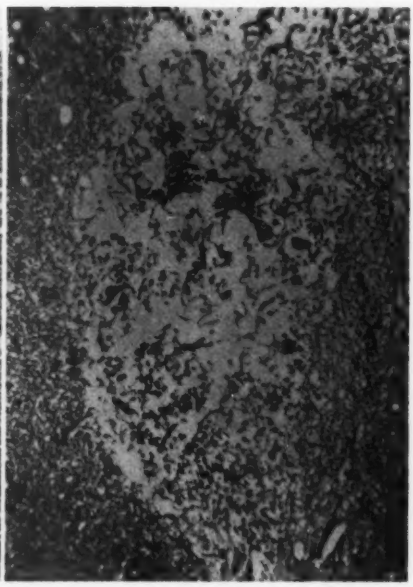

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PLATE 188

FIG. 31. Case 10. Deposit of calcium salts on periphery of patch of encephalomalacia in globus pallidum. The black rings are calcified blood vessels. × 85.

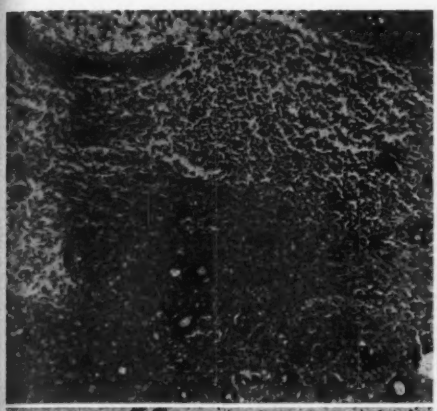
FIG. 32. Case 10. Large accumulations of calcium salts in thalamus. × 85.

FIG. 33. Case 10. Coarse, black granules of calcium salts shown by von Kossa's stain. Region of brain adjacent to that illustrated in Figure 32. × 85.

FIG. 34. Case 10. Multinucleated giant cell has engulfed several particles of calcium salts. Of note are the adjacent large, foamy macrophages. Globus pallidum. × 295.

FIG. 35. Case 10. Multinucleated giant cells, mononuclear phagocytes, and calcium salts in thalamus. × 295.

FIG. 36. Case 10. Complete loss of Purkinje cells and reduction in number of granular cells in cerebellar folium. Bergmann glia unaffected. × 85.



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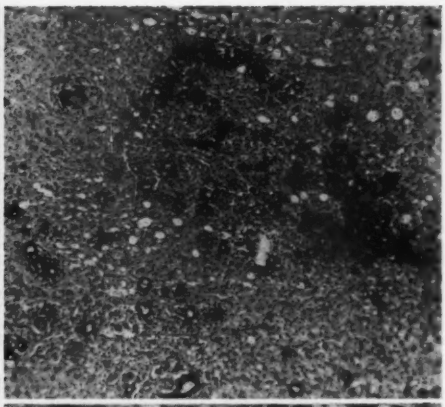

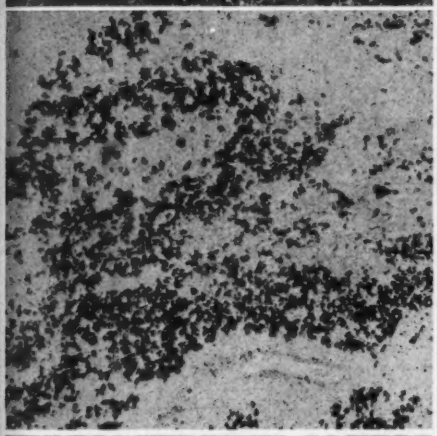

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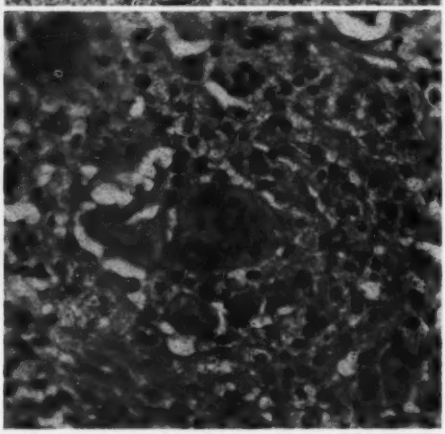

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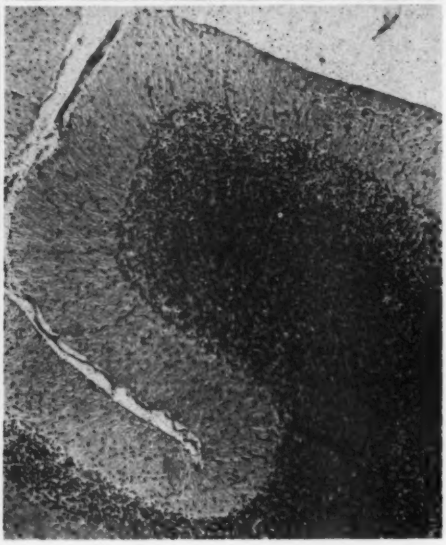

HUMAN SALMONELLOSIS DUE TO SALMONELLA SENFTENBERG *

THEODORE J. CURPHEY, M.D.
(From the Pathological Laboratories, Meadowbrook Hospital, Hempstead, N.Y.)

The recent rapid advances in the field of intestinal bacteriology, more especially as they concern the organisms of the Salmonella group, prompt the reporting of a case of intestinal infection with autopsy findings in which a rare strain, namely *Salmonella senftenberg*, was isolated.

REPORT OF CASE

History. L. G., a white male, 62 years of age, a waiter in a nearby country club, was admitted to Meadowbrook Hospital on December 30, 1944, and died on March 8, 1945. His chief complaint was diarrhea of 2 weeks' duration. He had been well previously, except for occasional attacks of indigestion. At the time of onset he developed severe and persistent vomiting and diarrhea, having as many as 50 watery, foul-smelling stools daily. During this time there had been marked weight loss. There was no history of jaundice, hematemesis, or melena.

Physical Examination. Physical examination showed a dehydrated, elderly white male. His tongue was very red. The lungs showed slight impairment of resonance on the right side posteriorly in the region of the third and fourth ribs. The breath sounds were generally diminished over both lungs. The heart was normal. Examination of the abdomen failed to reveal tenderness or organ enlargement. A left scrotal hernia was present. The extremities were normal.

Laboratory Data. Urine: Negative on two examinations, with presence of an occasional hyaline cast in another sample about 3 weeks prior to death. *Blood:* Four days after admission his hemoglobin was 105 per cent; red cell count, 5,000,000 per cmm.; white cell count, 12,000 per cmm.; neutrophils, 60 per cent; lymphocytes, 40 per cent. Four days later the hemoglobin was 112 per cent. A week later (2 weeks after admission) the hemoglobin was 76 per cent; white cell count, 4,000 per cmm.; neutrophils, 42 per cent; lymphocytes, 58 per cent. One month later the hemoglobin was 72 per cent; leukocytes, 5,000 per cmm.; neutrophils, 55 per cent; lymphocytes, 45 per cent. *Chemical Examination of the Blood:* On admission, nonprotein nitrogen, 72 mg. per 100 cc.; sugar, 130 mg. per 100 cc. Two weeks later, nonprotein nitrogen, 25 mg. per 100 cc.; creatinine, 1.5 mg. per 100 cc.; sugar, 85 mg. per 100 cc. Total serum proteins on this date were 3.3 per cent with 1.4 per cent albumin and 1.9 per cent globulin. The total cholesterol was 160 mg. per 100 cc. Total protein 1 month after the first determination and 6 weeks from the date of admission showed a total of 4.6 per cent with 2.5 per cent albumin and 2.1 per cent globulin. Five days before death the acid phosphatase determination was 3.7 King-Armstrong units; the alkaline phosphatase, 17.3 King-Armstrong units. Wassermann and Kahn tests gave negative results. Cephalin flocculation test performed 5 days before death showed a 1 plus reaction. Serum taken for Widal test on day of admission agglutinated *Bacillus typhosus* (alcoholic antigen) in a dilution of 1:80. When the test was repeated 1 month later, no agglutination with typhoid antigen was obtained. *Gastric Contents:* An analysis done 12 days after admission showed no free acid, no blood, total acid of 4° in the fasting specimen and 12° in the first hour specimen. The material was insufficient for further acid determination in the second, third, and fourth hour samples. *X-Ray Study:* A roentgenogram of the gastrointestinal tract, taken 2 weeks after admission, showed considerable dilatation of the small intestine by gas but no evidence of delay of the progress of the meal. The roentgenologist

* Received for publication, September 7, 1945.

interpreted these findings as evidence of spasticity of the small intestine. The barium meal showed also marked colonic stasis and a loop of large intestine in the scrotal sac.

Bacteriological Identification. A sample of feces obtained 6 days after admission was forwarded to the Salmonella Center of the New York State Department of Health Laboratories, at which time a preliminary report was given on the isolation of an organism of the Salmonella group. A subsequent final report stated that this had been determined to be *S. senftenberg.**

Clinical Course. On admission the patient's temperature was 97° F. Subnormal temperature persisted for the next day, but on the third hospital day the temperature reached 99° F. For the next 3 weeks it ranged for the most part in the subnormal zone; during the fourth and fifth week the temperature was normal; during the sixth and seventh week and part of the eighth week it was again subnormal, ranging between 97° and 98° F. The pulse rate varied between 70 and 80. Respiration rate ranged between 18 and 25 during the first week and between 15 and 20 subsequently. About 1 month after admission the patient had edema of both ankles and ascites. He was placed on a high protein diet, following which the serum proteins were determined and showed an appreciable increase over the previous test. An electrocardiogram taken 1 month after admission showed low voltage in all three leads. During a good part of the clinical course it was necessary to resort to tube feeding. The diarrhea appeared to be controlled, but recurred when refusal of food led to tube feeding. The stools were green, and during the first week of admission varied from one to two daily and continued so for the second and third weeks. On the fourth week they increased in frequency to three and four daily. On the fifth week the bowels moved once daily. During the sixth week the bowel movements averaged two to four daily. In the seventh and eighth weeks he had two stools daily. In the ninth week diarrhea was noted. During the last few weeks of his life the patient was apathetic, listless, and showed marked anorexia with increasing weakness. During the last 2 days of his life he had large dark brown stools, one or two daily. Immediately prior to death multiple purpuric areas were noted on the dorsa of the hands. He died on the 68th hospital day. Autopsy was performed 5 days later, the body having been unclaimed.

AUTOPSY FINDINGS

The body was markedly emaciated and poorly developed, measuring 5 ft. 10 in. in length and weighing approximately 160 lbs. The lips were dry and the tongue parched. No external evidence of lymphadenopathy was seen. There was marked atrophy of the subcutaneous fat. The abdomen was scaphoid. There was rather extensive scaling and hyperkeratosis of the skin, particularly over the legs. On opening the peritoneal cavity the structures were dry in appearance. The heart weighed 200 gm. The myocardium was unusually flabby and on section revealed a light grayish red surface. The coronary vessels were normal. The aorta showed minimal arteriosclerotic change. The lungs showed no noteworthy changes. The liver weighed 860 gm. The capsule was light brown and smooth, the lower edge sharp. The cut surface showed dark brown tissue in which the usual lobular markings were normal. The gallbladder was markedly contracted and contained

* I wish to express my appreciation to Dr. Ruth Gilbert and Miss Marion Coleman of the Division of Laboratories and Research of the New York State Department of Health for their valuable assistance in the identification of this organism.

several irregular calculi, the wall being thick and fibrotic. The spleen weighed 130 gm. The capsule was grayish red and smooth. On section the cut surface showed a soft, light grayish red pulp. The follicular markings were not visible. The knife scrapings were greatly increased. The esophagus was normal. The stomach was greatly distended with approximately 500 cc. of semi-solid food material and a large amount of gas. The rugal folds were greatly flattened. The mucosal surface was reddish brown. No ulcers were seen. Within the small intestine, particularly in the mid-jejunal area, numerous widely separated, minute, superficial, blackish red ulcers were found, measuring from 1 to 3 mm. in length. They were oval and appeared to be situated on the crests of the valvulae conniventes. The tissue was moderately hyperemic around many of the ulcers. A few ulcers were found also in the lower ileum. None was present in the proximal jejunum. The Peyer's patches were prominent but showed no evidence of ulceration. The ascending colon and sigmoid showed large, irregular, brownish black, shaggy ulcers with a pseudomembranous covering and measuring up to 7 cm. in diameter. In addition, there were several small, moderately deep, oval ulcers measuring 1 cm. in diameter. These grossly resembled those in the small intestine but were deeper and many of them presented sharply sloping edges. The uninvolved mucosa of the large gut showed a deep blackish blue discoloration. The regional mesenteric lymph nodes as well as the peripancreatic nodes were moderately and discretely enlarged, measuring up to 2 cm. in greatest diameter. On section they cut with less resistance than normal to reveal a pinkish red, homogeneous, edematous-appearing surface. The pancreas and adrenals showed no noteworthy changes. The right and left kidneys weighed 120 gm. each and showed no noteworthy gross change. The prostate was moderately enlarged and nodular. The left internal inguinal ring showed a large hernial opening through which the fingers could be passed into the scrotum. No bowel or omentum was present in this sac. Sections of bone showed normal appearing dark red marrow.

Anatomical Diagnoses. Acute and subacute ulcerative and pseudomembranous enteritis. Acute mesenteric and pancreatic lymphadenitis. Acute myocardial degeneration. Chronic cholecystitis with lithiasis. Acute splenic tumor. Hyperkeratosis of abdomen and extremities. Dehydration, avitaminosis (?).

Microscopical Findings

The pertinent histological changes were seen in the gastrointestinal tract and the regional lymph nodes. A typical lesion in the small intestine (Fig. 1) showed the shallow character of the ulceration of the

mucosa, the majority of the ulcers lying superficial to the muscularis mucosae. The mucosal and submucosal areas contiguous to the zone of ulceration were heavily infiltrated with mononuclear leukocytes (Fig. 2), many of the submucosal lymphatics being dilated and filled with these cells.

The lymph nodes from the mesenteric and peripancreatic areas (Figs. 3 and 4) showed interstitial edema and crowding of the sinusoids with many mononuclear leukocytes. The pericapsular fat was heavily infiltrated with mononuclear cells and the lymphatics immediately adjacent to the nodes were markedly dilated and packed with lymphocytes and mononuclear leukocytes (Fig. 5). At a higher magnification (Fig. 6) many of the mononuclear leukocytes showed macrophagic activity in which the cytoplasm of the cell was seen to contain particles of pyknotic-appearing basophilic débris. These cells were identical in morphological and tinctorial qualities with the macrophages seen in typhoid fever, and, indeed, many of the histological features observed in the ulcers of the intestine and in the lymph nodes were quite similar, if not identical, to those seen in that disease.

Bacteriological Study

The feces, as well as a representative ulcer of the intestinal tract, were saved for culture. From each of these *S. senftenberg* was isolated in the State Department of Health Laboratories, as well as in our own laboratory.

The organism was a gram-negative, nonmotile bacillus producing acid and gas in dextrose, maltose, and mannitol. It failed to ferment lactose or sucrose and did not produce indole. In Kligler's medium it produced acid with gas in the butt. The medium was blackened due to hydrogen sulfide production. Further cultural and serological study at the State Department of Health Laboratories established that it was a strain of *S. senftenberg.*

Discussion

With the pathological findings at autopsy mainly localized in the gastrointestinal tract, and the recovery before death of a species of Salmonella from the feces, it would seem probable that the organism was the cause of the ulcerative changes found in the intestinal tract. The clinical history of gastrointestinal symptoms with diarrhea would lend further credence to this belief and would thus make it reasonable to assume that this was a fatal case of relapsing subacute ulcerative enterocolitis caused in all probability by *S. senftenberg.* If these facts are acceptable, then this is the first reported fatal case in a human being caused by this species of Salmonella.

This organism has been encountered previously in veterinary and medical material. Edwards,[1] in 1937, isolated it in connection with an epidemic in turkeys. He called attention to the fact that the serological reactions established a similarity between it and the Jena and Dublin varieties of *S. enteritidis*. He found that the organism produced acid and gas in glucose, trehalose, arabinose, xylose, sorbitol, and dulcitol, but that lactose, sucrose, and inositol were not attacked. He further noted that hydrogen sulfide was formed and that prompt production of acid developed in tartrate agar. Edwards' cultures were found to be serologically identical with the Senftenberg type of Kauffmann[2] (1929) and the Newcastle type of Warren and Scott.[3] He stated that Kauffmann and Mitsui,[4] in 1930, demonstrated that the only difference in these types is the production of hydrogen sulfide by the Senftenberg strain and the failure of the Newcastle strain to produce it. It must be noted, however, that in Warren and Scott's report in 1929 they stated that a new serological type of Salmonella isolated by them and designated as Newcastle I and II did show very feeble production of hydrogen sulfide with slow and slight blackening of lead acetate medium. Edwards' strains were isolated from young turkey poults, showing icteric livers and joint involvement, with a low mortality rate (not exceeding 10 per cent) in the flock examined. It is of note that this report of Edwards is the first account in which *S. senftenberg* was definitely associated with a pathological process in man or animals.

In dealing with Salmonella infections in man, a recent report by Seligmann, Saphra, and Wassermann[5] details an analysis of 1,000 cases bacteriologically identified by the New York Salmonella Center. Of these 1,000 cases, *S. senftenberg* was isolated from only 9. The organism was recovered from the stool in each case and was obtained in five outbreaks of disease, all coming from a limited episode in an asylum in Massachusetts. Furthermore, of these 9 cases, 4 patients were healthy carriers, the other 5 showing symptoms of gastro-enteric disease. In none of these 5 cases was there a fatal outcome. In 1942, Bornstein and Saphra,[6] in a report of certain unusual Salmonella types isolated by them, stated that this organism (*S. senftenberg*) had previously been found by them in Chinese eggs and had also been isolated from a human carrier. In 1943, Edwards and Bruner[7] examined 3,090 cultures of Salmonella isolated from 2,285 outbreaks of infection in man and animals. *S. senftenberg* was isolated from 15 outbreaks in fowls, 4 outbreaks in swine, and 4 outbreaks in man. Furthermore, of the 10 cultures of *S. senftenberg* isolated in man during this study, 8 were recovered from normal carriers and 2 from cases showing symptoms of gastro-enteric disease. Neither of these 2 cases was fatal. From the above evidence it can be seen that the organism is a rare

invader when compared to the other Salmonella types. It shows, too, that it is often isolated from human carriers showing no evidence of disease.

This ability to recover Salmonella from human carriers makes it necessary to exercise caution in imputing a causal relationship between any pathological process found at autopsy and a Salmonella strain recovered either before death or from autopsy material. Thus, Seligmann, Saphra, and Wassermann [5] stated: "The finding of Salmonella in a fatal case does not of necessity imply that the recovered organism is the sole or even a contributory cause of death." It appears, however, from a study of the clinical course in my case, coupled with the autopsy findings, that no other demonstrable cause of death was determined and that the main pathological changes were found in the gastrointestinal tract. The recovery of the organism from the stool during life and at autopsy from the fecal contents and intestinal ulcers would seem to warrant the conclusion that the organism was pathogenic for this host and was the cause of the ulcerative lesions. While too much significance cannot be attached to the fact that the mucosa of the intestinal tract showed marked hydrogen sulfide staining because of the length of time between death and autopsy, nevertheless the knowledge that *S. senftenberg* is a hydrogen-sulfide-producing organism might be adduced as confirmatory evidence that the organism can be incriminated as the etiological agent responsible for the ulcerative lesions found in the intestine.

While it is hazardous to attempt to outline the pathogenesis of *S. senftenberg* infection in the human being from the study of a single case, nevertheless the clinical history and the gross and microscopical findings in this case seem sufficiently similar to those of certain well established enteric diseases to warrant the attempt. Thus it is obvious from the gross and microscopical studies that this infection is essentially like that seen in paratyphoid fever caused by *S. paratyphi* and *S. schottmülleri*, respectively. For instance, the gross distribution of the ulcerative change in the small and large intestine, with pseudomembranous inflammation, is reminiscent of paratyphoid fever in contrast to the greater tendency to localization of ulceration in the small intestine in typhoid fever. In addition to the distribution of the lesions is the fact also that grossly and microscopically, *S. senftenberg* infection is quite like that of paratyphoid fever in that the ulcers are superficial, being confined for the most part to the mucosal layer and the upper layers of the submucosa, with the characteristic mononuclear cellular exudate seen in these typhoid-like infections of the gastrointestinal tract. The dense mononuclear cellular infiltration of the

lymphatics and the diffuse mononuclear proliferation with evidence of phagocytosis in the regional lymph node also conform to this picture.

It is of interest to consider the relationship between the clinically observed protracted gastro-enteric disease with periods of acute exacerbation and remission and the pathological findings. Thus the gross inspection failed to reveal areas in the small or large intestine that could be interpreted as evidence of healed ulceration as is sometimes seen in infections with closely allied organisms, *e.g., Bacillus typhosus.* If the ulcers seen at autopsy were typical of those present during the previous exacerbations as suggested by the periodic attacks of diarrhea, their very superficial nature would suggest that complete resolution of the mucosa occurs during the period of remission, only to be followed by fresh areas of ulceration during a relapse in the disease. There is thus no controvertible evidence in the material here submitted that *S. senftenberg* in the human being does not behave exactly like other known Salmonella infections. Moreover, this strong resemblance might be adduced as sound evidence that the organism isolated from the feces and ulcers during life or at autopsy was in all probability the true inciting agent of the pathological changes present in this case.

Another point of general interest in this case is the fact that the patient was a food-handler who could well have harbored the organism for many years prior to the actual symptoms of his disease. *S. senftenberg* is more often found in normal human carriers than as a disease incitant and sporadic outbreaks of Salmonella infections are frequently due to food-handlers who are carriers of the organism.

In conclusion, it would seem that the importance of the correct identification of intestinal incitants of disease has not received sufficient emphasis. For example, it is the general practice to terminate the routine bacteriological investigation of submitted fecal material after the usual cultural and serological approaches have been made towards the identification of the common incitants found in the typhoid-colon-dysentery group. Frequently an organism having cultural features that do not readily place it in this large group is not studied in greater detail because its possible significance in the causation of the intestinal disease is under-estimated. It is this type of organism that should receive more extended study in the hands of an experienced laboratory group working with strains of Salmonella. The very multiplicity of the Salmonella types would tend to emphasize the need for accurate identification by special technical approaches, if the epizoology, epidemiology, and control of salmonellosis are to be the subjects of serious study. Furthermore, the experience in this single case would lead one to suspect that many of the types of Salmonella that

are now established as not being pathogenic for man might be found to be disease incitants if more detailed cultural studies were made on clinical and autopsy specimens by those specially skilled in isolating and identifying these organisms. With this in mind, it would seem desirable to study by cultural methods all cases which at autopsy present inflammatory gastrointestinal lesions. Whenever an organism is recovered which cannot be readily identified, it should be submitted to a Salmonella center for further study.

Summary

1. What is believed to be the first reported example of fatal human intestinal infection by *Salmonella senftenberg* is described. The organism was isolated during life and at autopsy from a patient with recurrent diarrhea.

2. Emphasis is placed on the importance of more detailed investigation of organisms of the enteric group having atypical cultural and serological features when such are recovered during life or at autopsy from patients showing gastro-enteric symptomatology or lesions.

3. Any organism recovered during life or at autopsy from cases of enteric disease and not readily identifiable by ordinary laboratory procedures should be forwarded to one of the Salmonella centers for investigation and identification.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

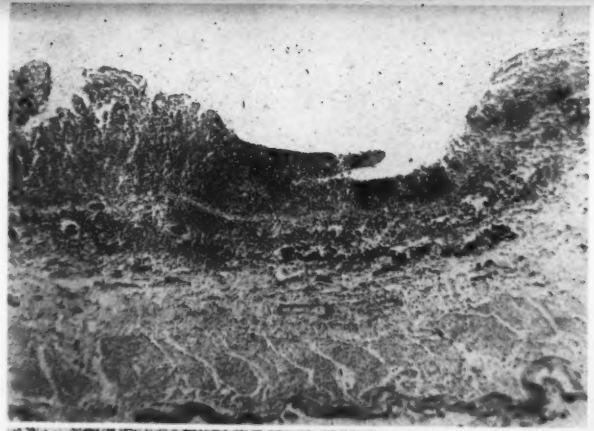
PLATE 189

FIG. 1. Section of an ulcer of the small intestine showing its superficial location above the level of the muscularis mucosae. The pattern of the adjacent mucosal gland is lost due to marked post-mortem autolysis. Hematoxylin and eosin stain. × 8.

FIG. 2. Higher magnification of Figure 1 to show the mononuclear cellular infiltration of areas contiguous to the zone of mucosal ulceration. A dilated submucosal lymphatic filled with these cells is seen in the center of the field. Hematoxylin and eosin stain. × 20.

FIG. 3. Lymph node densely packed with mononuclear cells with associated fibrous thickening of the capsule and dense pericapsular mononuclear cellular infiltration. Hematoxylin and eosin stain. × 15.

1



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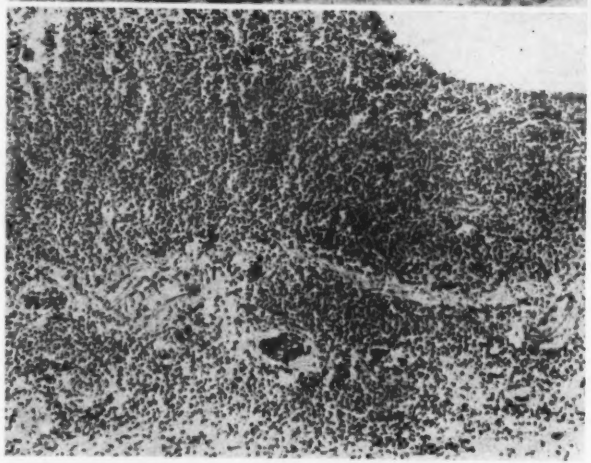
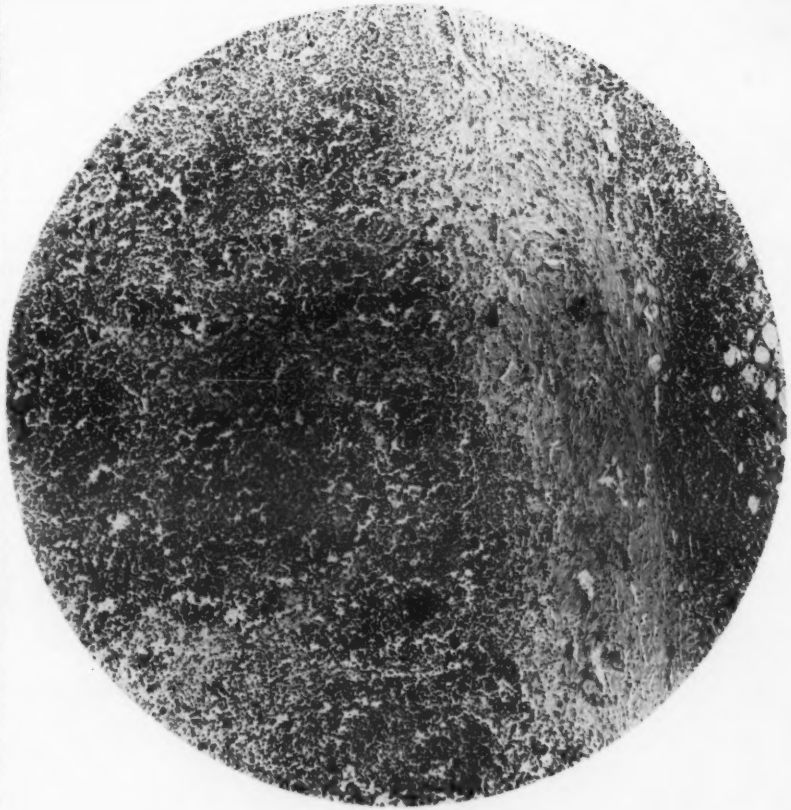


Curphey



3

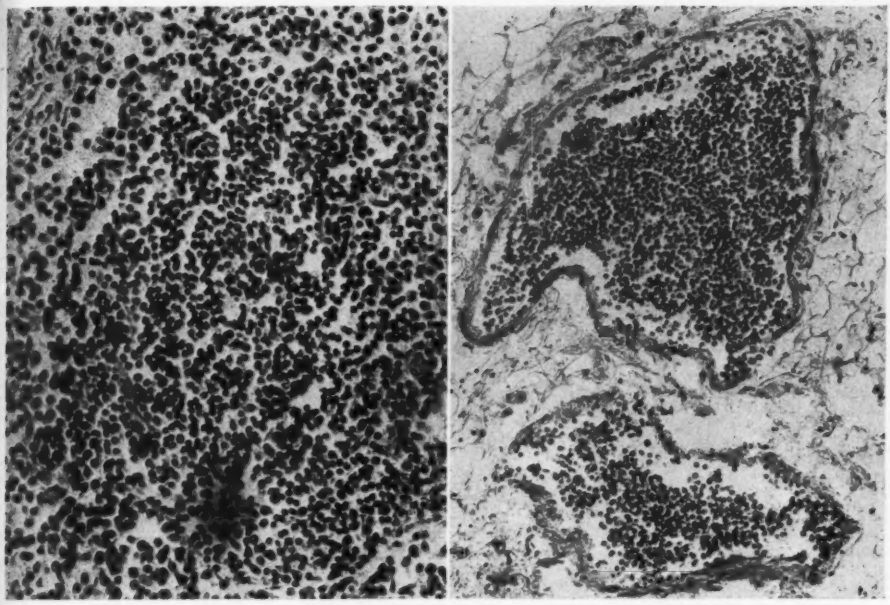
Salmonellosis

PLATE 190

FIG. 4. Higher magnification of Figure 3 showing a well preserved lymph follicle surrounded by an edematous reticulum rich in lymphocytes and mononuclear leukocytes. Hematoxylin and eosin stain. × 180.

FIG. 5. Perinodal fat containing dilated lymphatics packed with lymphocytes and mononuclear leukocytes. × 25.

FIG. 6. Higher magnification of Figure 4 showing in the upper left-hand portion of the field the presence of typical macrophages with phagocytized débris. Hematoxylin and eosin stain. × 400.



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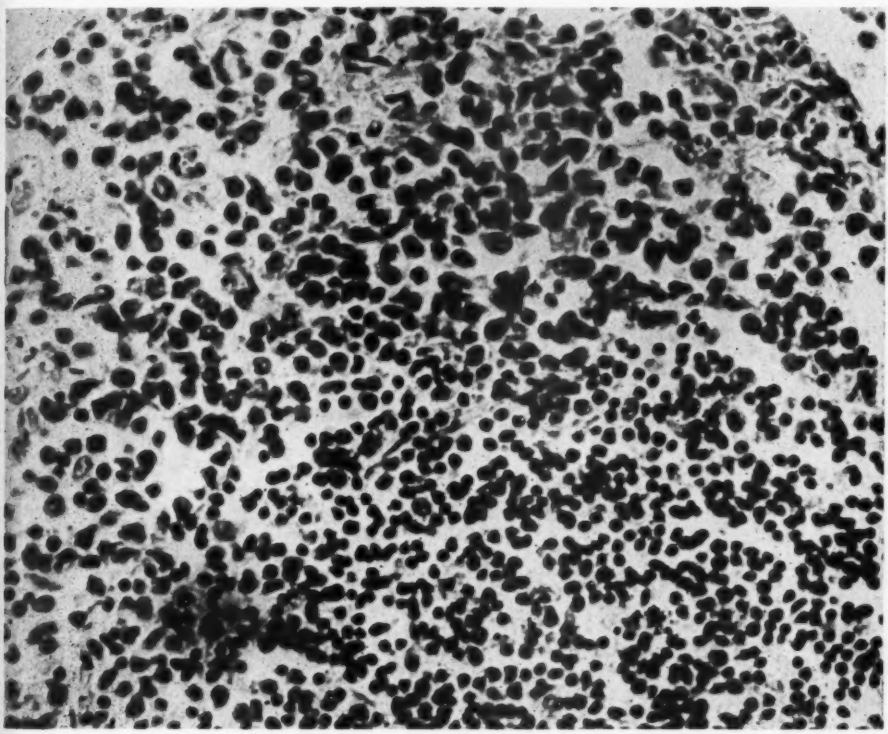

Curphey Salmonellosis

ARTERIAL CALCIFICATION IN INFANCY WITH SPECIAL REFERENCE TO THE CORONARY ARTERIES *

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Unlike occlusive lesions of the coronary arteries of older children and adults, which may result from any one of several diseases,[1] occlusion of these arteries in infants is nearly always due to a single type of arterial disease. This lesion consists of calcification of the wall of the artery with fibroblastic proliferation of the intima. The intimal proliferation may be so marked as eventually to cause complete or nearly complete obliteration of the lumen. This lesion may occasionally be found in the arteries of older persons but its comparative significance in that age group is lessened by the more frequent occurrence of arteriosclerosis of the atheromatous type, inflammatory processes, and other arterial diseases.

Fifteen cases of calcification of the coronary arterial wall with intimal proliferation in infants have been previously reported. These cases are collected in Table I. In some instances this involvement of the coronary arteries has been the cause of death; in others, similar involvement of other vessels has been fatal. The ages of the 15 patients ranged from 1 day to 27 months. Seven were male and 8 female. In all cases, other arteries were involved in addition to the coronary arteries. Five children died suddenly, while others had presented varying clinical evidences of cardiac failure.

In the Department of Pathology of the University of Michigan the hearts of 4 infants with calcification of the coronary arteries have been examined. A fifth case was seen in the pathological service of the Providence Hospital, Detroit. A description of that case is included by the courtesy of Dr. Donald H. Kaump. In one case the lesion was severe enough to be the probable cause of death, while in 3 cases it was a possible contributing cause. In the other case only earlier stages of the process were shown.

REPORT OF CASES

Case 1

A male infant, 3 months old (7220-LAI), had been considered ill for only 3 days. Clinical symptoms were described as those of an "influenzal pneumonia." One male sibling was said to have died with similar symptoms. No history was obtained of unusual dietary or other features for either the child or its mother.

Autopsy was performed at another hospital and only portions of

* Received for publication, October 2, 1945.

TABLE

Summary of Reported Cases of

Case no.	Author	Year	Age	Sex	Renal lesions	Other lesions	Parathyroids
1	Bryant and White[2]	1891	6 mos.	M	Hydronephrosis		
2	Surbek[3]	1917	3 days	F	Chronic nephritis	Pericarditis; splenomegaly	
3	Verocay[4]	1920	5¾ mos.	F	Focal calcification in renal glomeruli	Congenital lues(?) (no spirochetes demonstrable)	
4	Hughes and Perry[5]	1929	7 wks.	F	No gross abnormality		
5	Forrer[6]	1930	3 mos.	M	Low-grade chronic glomerulonephritis	Navel infection; infectious splenic tumor	
6	Iff[7]	1931	1 day	M	Glomerular and intertubular calcification		Normal
7	Lightwood[8]	1932	27 mos.	F	Glomeruli fibrotic and calcified; interstitial fibrosis; epithelial calcification	Bronchopneumonia; epiphyseal lines broad, irregular; osteoporosis and osteosclerosis	One identified; normal; no evidence of tumor in neck
8	Oppenheimer[9]	1938	6 mos.	F	Calcification of arteries with early scarring	Bones normal	Small; no microscopic lesion
9	Oppenheimer[9]	1938	4 mos.	F	Calcification of arteries with calcification of glomeruli and tubules	Bones normal	Not examined
10	Brown and Richter[10]	1941	3 mos.	M	Swelling of glomeruli and of epithelium of tubules; calcification of arteries		Not examined
11	van Creveld[11]	1941	6 wks.	F	Early glomerulonephritis		
12	van Creveld[11]	1941	9 wks.	M	Early glomerulonephritis		
13	Baggenstoss and Keith[12]	1941	8 wks.	F	Arteriosclerosis of kidney; increased endothelial cells in glomerular capillaries; some hyaline and calcified glomeruli; "no evidence of severe renal disease"		
14	Andersen and Schlesinger[13]	1942	6½ mos.	M	Bilateral hydronephrosis	Early renal rickets; acute lobular pneumonia	5 times normal size; all 4 glands examined
15	Andersen and Schlesinger[13]	1942	5 mos.	M	Polycystic kidneys	No definite rickets by roentgenogram	One examined was 5 times normal size
16	Stryker	1946	3 mos.	M			Not examined
17	Stryker	1946	2 mos.	F	Calcification of tubular epithelium	Acute purulent bronchitis and bronchopneumonia	Not examined
18	Stryker	1946	6 mos.	M		Early acute lobular pneumonia	Not examined
19	Stryker	1946	Stillborn	M	Polycystic kidneys		Not examined
20	Stryker (Kaump)	1946	7 mos.	F	Calcification of arteries	Occasional foci of acute pneumonitis; focal fibrosis and calcification of myocardium; fibrosis of endocardium of left ventricle	Not examined

organs were available for microscopic examination. Gross findings were not noted. Microscopic examination of the coronary arteries showed heavy calcification of the internal elastic membrane and adjacent inner portion of the media (Fig. 2). Central to this calcification the lumen

I
Coronary Arterial Calcification in Infants

Vitamin D	Syphilis	Other factors	Congenital abnormalities	Family history
			Congenital urethral dilatation	
	Wassermann of mother negative	Sudden death		Mother: developed otitis on third puerperal day; low-grade infection during pregnancy(?)
	"Congenital lues"; Wassermann positive (1 plus)	Given antiluetic therapy; clinical rickets	Hydrocephalus(?)	Syphilis
	Wassermann negative	Sudden death		Mother: had "influenza" during sixth month of gestation
	Wassermann negative			Sibling died suddenly at 6 months
	Wassermann of mother negative	Hydramnion	Abnormal course of aortic arch	Mother: past history of nephritis; two normal older siblings
"In physiological quantities only"	Wassermann negative	Albuminuria; high blood pressure; calcium, 11 mg. %; phosphorus, 6.68 mg. %; fever		
		Sudden death		
		Cardiac failure		
Given "in normal amount"	Kahn of mother negative	Illness of 24 hours' duration		Mother and father: healthy
None	Wassermann of parents negative			Mother: asthmatic; use of lobeline and caffeine during pregnancy; 1 prior abortion
				Mother: eczema; family history of asthma
None(?)		Sudden death		Mother: well during pregnancy
4 times usual dose	Wassermann negative	Calcium, 7.5 to 11 mg. %; phosphorus, 6 to 10 mg. %; nonprotein nitrogen, 55 to 120 mg. %; "otitis media"; death not sudden	Congenital hypoplastic kidneys	7½ months premature sibling died on first day; no familial disease
Given in moderate amounts		Calcium, 6.5 to 9 mg. %; phosphorus, 6.4 to 11.3 mg. %; sudden death		Family history irrelevant
None				Male sibling died with similar symptoms
	Congenital lues; Kahn, 4 plus	"Parotitis"		Syphilis(?)
	No serologic test	"Snuffles"		One miscarriage; sibling died at 5 weeks, cause unknown; one sibling in good health
	Kahn of mother negative		Multiple anomalies	Mother: bronchiectasis; allergic bronchitis
Cod-liver-oil concentrate; 2 drops daily during first month	Serologic test of mother negative	"Chronic cold" for 3 months		"Normal pregnancy"; "family history not contributory"

of one artery was completely occluded by young connective tissue in which there were many newly formed vascular channels. Other main coronary stems showed a partial occlusion of the lumina by a similar process. In this young connective tissue there was an occasional poly-

morphonuclear leukocyte or lymphocyte. No hemosiderinophages were seen. The appearance of the connective tissue was uniform. In the adventitia there was an infiltration of polymorphonuclear leukocytes, lymphocytes, and monocytes; this infiltration was more marked focally but the distribution was not perivascular. Sections stained with van Gieson's stain for connective tissue and with the Verhoeff stain for elastic tissue demonstrated this newly formed tissue to be young fibroblastic connective tissue.

The myocardial fibers were considered to be slightly hypoplastic. In the myocardium were small foci of recent infarction, located chiefly in the subendocardial area and in the papillary muscles (Fig. 1). The finer branches of the coronary arteries appeared normal. Subendocardial vacuolar degeneration was present. Changes in other organs were largely limited to the vessels. The lungs showed acute passive congestion and acute edema. In a large pulmonary artery there were small scattered foci of degeneration and calcification of the wall, and, in smaller branches, focal calcification of the internal elastic membrane. Arteries at the hilum of the spleen and in the pelvis of the kidney (Fig. 3) showed calcification and partial occlusion similar to that in the coronary arteries. The splenic vein contained a recent thrombus and the spleen showed acute passive congestion.

Pathological Diagnoses. Marked calcification of the walls of the coronary arteries with associated connective tissue proliferation of the intima; complete occlusion of a main coronary artery; recent myocardial infarcts; calcification and intimal proliferation in splenic, renal, and pulmonary arteries; recent thrombus in splenic vein; acute passive congestion of all viscera; acute pulmonary edema; patchy pulmonary atelectasis and emphysema; cloudy swelling of liver and kidneys.

Case 2

A female infant, 2 months old (A-268-AJ), was admitted to the hospital because of prematurity. She had been born after 7 months' gestation and at the time of admission weighed only 3 pounds. The delivery had been normal. Slight cyanosis had been noted at birth. She was treated in the hospital as a feeding problem. A routine Kahn test of the blood gave a 4 plus reaction; the result of a second Kahn test was similar. The only family history available was that both parents were living and well; there were no siblings. Antileutic therapy was started. The patient did not gain weight satisfactorily. Additional complications were diarrhea of unexplained cause and a recurrent elevation of the temperature to 102° F. Despite various therapeutic measures she died after 2 months of hospitalization. Clinical diagnoses were prematurity and congenital syphilis.

No lesions were noted on gross examination of the heart. On microscopic examination, however, the coronary arteries showed calcification of their walls of varying extent. In some arteries only a fine basophilic stippling could be seen along portions of the internal elastic lamina,

either on the membrane or closely applied to its margins. In other areas the amount of deposit was greater; one or both sides of the membrane might be affected. The calcification did not involve the entire circumference of the arteries but was present in patchy areas. The internal elastic lamina maintained its identity as an eosinophilic strand through the calcium except for occasional interruptions of its continuity where calcification was most marked. In some of the foci of early deposition of calcium the lamina was basophilic. Over the patchy areas of calcification there was early fibroblastic proliferation of the intima. This did not appear to be sufficient to cause occlusion of the artery but there was diminution of its lumen. No lesions were found in more distal portions of the arteries, nor were lesions seen in the myocardium.

The splenic artery showed a localized area of similar calcification, with calcification also in the adjacent fibroblastic tissue of the intima. The elastic lamina was seen as an eosinophilic band in the calcareous material (Figs. 4 to 6). Fibroblastic proliferation in the intima was not marked. Other significant findings were early purulent bronchitis and bronchopneumonia, and fairly marked calcification of some of the epithelium of the convoluted tubules of the kidneys. A presumptive Kahn test on the heart's blood was positive although the routine Kahn test was negative. There were no histologic evidences of syphilis in any of the tissues.

Pathological Diagnoses. Prematurity; early purulent bronchitis and bronchopneumonia; calcification of coronary arteries with early fibroblastic proliferation of the intima and reduction of the lumen without occlusion; localized calcification of the wall of the splenic artery; focal calcification of the renal tubular epithelium; congenital syphilis (?) (positive Kahn reactions).

Case 3

A colored male infant, 6 months old (A-66-T), was hospitalized because of difficulty in feeding associated with retraction of the head, present since birth. The infant had been born at full term. For the first 7 weeks of life jaundice was said to have been present but this disappeared. The mother had had one miscarriage at 4½ months; a sibling had died at 5 weeks of age of a cause not known, and another sibling, 4 years old, was in good health. The patient had had no acute illness, but "snuffles" had been present for about 8 weeks. During hospitalization the infant frequently refused feedings and became increasingly weaker. He died on the second day after admission. No serologic tests were performed.

At autopsy, all organs showed marked atrophy. Microscopic examination of the heart showed changes in the coronary arteries similar to those described in the previous cases. The deposition of calcium was again along the course of the internal elastic lamina. There were frequent interruptions of the continuity of the lamina. At one level the

fibroblastic proliferation of the connective tissue of the intima was sufficient to decrease the lumen of the vessel (Fig. 7). No myocardial lesions were found.

An artery in periadrenal connective tissue showed a more advanced stage of the lesion (Fig. 8). The internal elastic membrane could not be identified, being included in the areas of calcification, and proliferation of the intima was so great that only a small blood-channel remained. Small giant cells of foreign body type were adjacent to some of the masses of calcium. In the splenic artery there was a localized area of calcification of the wall without intimal change. The internal elastic lamina was included in the area of calcification and showed interruption in continuity.

Other significant findings were chronic passive congestion of the lungs with many hemosiderin-filled macrophages and chronic passive congestion of the liver. Early lobular pneumonia was present. Adipose tissue showed marked atrophy.

Pathological Diagnoses. Calcification and fibroblastic intimal proliferation of coronary arteries and of periadrenal and splenic arteries; chronic passive congestion of lungs and liver; early lobular pneumonia; malnutrition with atrophy of all tissues.

Case 4

A stillborn male infant (A-179-AL) was delivered after 8 months' gestation. The mother, 14 years old, suffered from chronic pansinusitis, allergic bronchitis, and bronchiectasis. Her Kahn test was negative.

At autopsy, the infant showed multiple developmental anomalies. Sections of the heart showed focal calcification of the wall of one coronary artery near its origin. The internal elastic lamina could be identified at either end of the mass of calcium, but was interrupted in the area of calcification. There were multiple small foci of necrosis and calcification in the myocardium. The lesion in the coronary artery was not occlusive and it is probable that the lesions in the myocardium did not result from it. Focal calcification on both sides of elastic fibers was found also in the pulmonary artery (Fig. 9) and in the first portion of the aorta.

Pathological Diagnoses. Stillbirth; multiple disturbances of development: encephalomeningocele, polycystic kidneys, polydactylism; focal calcification of a coronary artery, the aorta, and the pulmonary artery; focal necrosis of the myocardium.

Case 5

A female infant, 7 months old, had been ill for about 3 months with a "chronic cold." The mother had had a normal pregnancy. The infant had been breast-fed; she had received 2 drops of a concentrate of cod-liver oil twice daily during the

first month. There had been no other illnesses. Her condition appeared to be improving, but 2 days before admission to the hospital an elevation of temperature was noted. Two days later her parents noted gurgling sounds in her throat; there was a further rise of temperature and more marked cough. She became cyanotic and was taken to Providence Hospital, Detroit, where she died before any examination could be made. Serologic test of the mother was negative for syphilis. Post-mortem roentgenologic examination showed marked cardiac hypertrophy.

At the autopsy performed by Dr. Donald H. Kaump, the heart weighed 100 gm. The mitral valve appeared slightly thickened, and the endocardium of the left ventricle was pale gray. There was slight, bilateral hydrothorax and gross evidence of purulent lobular pneumonia, confirmed microscopically.

The striking lesions, however, were found in the cardiovascular system. The small arteries and arterioles of the heart, lungs, kidneys, pancreas, and periadrenal adipose tissue showed varying degrees of calcification involving and adjacent to the internal elastic membrane, with proliferation of the connective tissue of the intima (Figs. 10 to 12). A vessel in the peripancreatic tissues was completely occluded except for newly formed vascular channels; a large coronary artery was partly occluded. Study of various vessels indicated that the first change was in relation to the internal elastic membrane, especially on the medial side, with subsequent intimal proliferation and involvement of the entire area previously occupied by the fibers of the media. That the deposited material was lime salts was indicated by the use of dilute hydrochloride acid on sections. Some of the muscle fibers of the media showed hyaline change. In some vessels a foreign body giant cell reaction to the calcareous masses was seen. The endocardium was thickened by proliferation of fibrous tissue. In the myocardium were scattered foci of fibrosis and in some of these calcification had occurred. Remaining myocardial fibers showed hypertrophy. Renal changes were limited to lesions in the arteries and arterioles.

Pathological Diagnoses. Calcification of the wall and fibroblastic proliferation of the intima of coronary, pulmonary, renal, pancreatic, and periadrenal arteries and arterioles; cardiac hypertrophy; fibrosis of the endocardium of the left ventricle; focal myocardial fibrosis with calcification; bilateral pulmonary edema; bilateral hydrothorax; bilateral acute purulent lobular pneumonia.

Discussion

The essential pathologic lesion in each of these cases is the same; the varied histologic pictures depend upon the stage and degree of the process. The arterial calcification occurs in relation to the internal elastic lamina, and its pattern is determined by the course of that membrane. This relation is shown by study of the earliest lesions;

in the later stages the lamina may be destroyed and its significance no longer apparent.

The various processes which may occur in the course of calcification of coronary and other arteries in infants include the following (not all may be observed in any one case): (1) The normally eosinophilic internal elastic lamina is interrupted by regions in which there have been deposited fine basophilic granules of calcium salts. (2) The internal elastic lamina may preserve its continuity as an eosinophilic hyaline band, with a fine deposit of basophilic material either on both sides of and closely apposed to it, or on the medial side only. (3) A loss of continuity of the internal elastic lamina is first observed where calcareous granules are in contact with it. (4) The internal elastic lamina may maintain its identity and be seen as a continuous or interrupted eosinophilic membrane either on the inner (intimal) border of deposited calcium salts or coursing through the midst of a large basophilic mass. (5) The internal elastic lamina may not be identifiable and only large clumps or broad bands of calcium salts be seen. (6) The larger calcific masses on the medial side of the internal elastic lamina occupy the area formerly filled with the muscular and elastic fibers of the media. It is believed that these fibers atrophy and are replaced by the deposits of lime salts. (7) An intimal fibroblastic reaction is present, beginning with the first fine basophilic deposits. Occasionally the calcific process in the vicinity of the internal elastic lamina extends into this fibrous tissue. (8) A giant cell reaction, of foreign body type, may be seen around some of the masses of lime salts.

Since the internal elastic lamina is morphologically a part of the intima of the artery, it may be questioned whether the term "medial calcification" should be applied to this process as most authors have done. When the calcareous material is deposited along the medial aspect of the lamina it may simulate a deposit in the media. This appearance is interpreted to be due to a previous atrophy of medial fibers and replacement of them by masses of calcium salts originating along the internal lamina. In nearly all previously reported cases of coronary calcification in infants, this relation of the calcium deposits to the internal elastic lamina, in at least the early stages, has been described.

This report is concerned chiefly with the process of calcification as it affects the coronary arteries. It should be noted, however, that these arteries are not the only vessels affected. In nearly all previously reported cases and in the 5 described here, there were arteries in other organs which showed lesions more or less severe than those in the heart. In some cases the effects upon organs supplied by these ves-

sels have been of principal clinical importance. Instances of calcification of similar type in the arteries of infants in whom the coronary arteries were not involved have been reported.[9] Probably the causal factors are similar.

Possible Causal Factors

The similarity of the vascular lesions and marked calcification in this type of infantile coronary calcification to those found in lesions known to occur in diseases with abnormal concentrations of serum calcium and phosphorus suggests possible causes.

1. Renal Lesions. In several previously reported cases renal lesions have been found. In some instances these lesions have been marked and of undoubted long duration. It is probable that these cases are actually examples of so-called calcium gout or of renal dysfunction with an altered calcium-phosphorus ratio and subsequent metastatic calcification. The term "renal rickets" has been applied to this disorder, although the osseous lesions of rickets have in some instances been minimal or lacking. Microscopic study of calcified arteries in other cases of renal rickets [14,15] has shown the calcification to occur in relation to the internal elastic lamina in early lesions and later to involve apparently the entire media. Thus the process is similar to that described in this report. Renal lesions of extent sufficient to consider them a primary factor in arterial calcification were present in cases reported by Bryant and White,[2] Lightwood,[8] and Andersen and Schlesinger,[13] and it is probable that the cases of Surbek,[3] Forrer,[6] and van Creveld [11] should likewise be included. The description of the renal lesions in these latter cases is incomplete. In 3 cases of the first group (Lightwood,[8] Andersen and Schlesinger [13]) in which chemical studies of the blood were made, abnormal calcium-phosphorus values were found. In case 4 of the present series, polycystic kidneys were present; this infant was stillborn, and the significance of such renal lesions in the extremely young has not been established.

The calcific lesions found in the kidneys in others cases may well be only another manifestation of the process causing the general arterial changes. In most of the instances in which calcification of tubular epithelium or of glomeruli has been found, there has been calcification also of the arteries of the affected kidneys. Unfortunately, no chemical studies of the blood are available for this group so that the degree of renal dysfunction is not known with certainty. That renal dysfunction is not the only etiologic factor, however, is shown by the occurrence of calcification in patients in whom no renal lesions were present.

2. Lesions of the Parathyroid Glands. Altered concentrations of

serum calcium and serum phosphorus may be due also to primary parathyroid hyperplasia or to a parathyroid tumor. Excluding those cases in which parathyroid hyperplasia is probably secondary to a renal lesion, the so-called renal hyperparathyroidism, the state of these glands is known only in the case reported by Iff.[7] No abnormality was found in that case. The morphologic appearances and the distribution of the calcific lesions in some cases of coronary calcification in infants are very similar to those in known cases of primary parathyroid hyperfunction. It is unfortunate that the parathyroid glands were not examined in the earlier cases, or in those reported here.

3. Primary and Destructive Diseases of Bone. Primary bone diseases, such as osteogenesis imperfecta, and destructive bone diseases, such as may occur with neoplastic metastasis, may be associated with altered concentrations of serum calcium and phosphorus. Calcification of arteries other than the coronary arteries has been reported in osteogenesis imperfecta [16] and in generalized osteitis fibrosa.[17] The bones were examined in only 3 of the cases of infantile coronary calcification reported in the literature. Changes were found in only one case [8] where the renal lesion and associated parathyroid hyperplasia furnished an explanation for the abnormalities. The few sections of bone available in my cases showed no change.

4. Hypervitaminosis D. A fourth cause of calcific lesions in arteries and tissues is hypervitaminosis D. This condition is also associated with altered serum values of calcium and phosphorus. Vitamin D was given to four of the infants whose lesions have been reported elsewhere. In 2 cases the amount was not excessive; [8,10] in 2 (Andersen and Schlesinger [13]) the dosage was larger than usual, up to four times the regular amount. The administration of vitamin D, however, is denied in many reports while other cases antedate the introduction of this product. No vascular lesions have been seen in many instances in which huge dosages have been given.[18] The experimental work of Goormaghtigh and Handovsky [19] with dogs indicates that the initial lesion in the calcification associated with hypervitaminosis D is degeneration of smooth muscle of the media. Such calcification is dystrophic in type. An increased sensitivity to a mild excess of vitamin D or calcium in cases of renal rickets may explain the frequency with which calcification is seen in that disease,[20] and it may be that in some persons there is an abnormal response even in the absence of renal lesions. In case 1 of the present series overdosage with vitamin D was originally suspected as a cause of the lesion, but the physician in charge was unable to find any known use of vitamin D in the family.

5. Infection. Factors other than altered calcium-phosphorus bal-

ance must be considered. Damage of the arteries may be the result of infection. Degeneration of the media, which may be severe enough to include necrosis and destruction of elastic fibers, has been reported [21] to follow certain toxic or infectious diseases, and calcification of the internal elastic lamina has been noted.[22] In 3 cases (Surbek,[3] Forrer,[6] and Lightwood [8]) there were coincident infective lesions of various organs, and Iff [7] believed that the mother of his patient had a low-grade infection during the period of gestation. In 3 of the cases here presented purulent pneumonitis was found, but in each this was a terminal feature and therefore probably was not related to the vascular lesions. The evidence in favor of infection as the primary cause in all cases of arterial calcification is slight, and there are multiple instances of intrauterine and neonatal infection without coexistent or subsequent arterial calcification. In some cases, however, it may be a contributing factor. Syphilis has also been implicated. In 2 of the 20 cases a diagnosis of syphilis was made either as a result of serologic examination of the patient or of the mother, or on the basis of tissue changes. In 10 cases, serologic examinations gave negative results. Again the evidence does not include all cases, but calcification is not a process generally considered to result from syphilitic infection. Search for spirochetes in the cases in which a diagnosis of congenital syphilis had been made was not successful.

6. Allergy. Allergy as the cause of arterial lesions was suggested by van Creveld.[11] He reported 2 cases, in the first of which the mother was asthmatic and had used caffeine and lobeline during the period of gestation. The suggestion was made that the lesions in the infant could be attributed to these drugs with probable damage to the vessel wall. Proof was attempted by animal experimentation, but there was no success. In his second case there was a familial history of allergic disease.

7. Other Nonspecific Calcific Lesions of Arteries of Similar Pattern. The dystrophic calcification of arteries in the senile uterus appears first in relation to the internal elastic lamina, but the arteries affected possess a thick hyalinized intima and there is occasionally fibrous tissue in the media. Such changes were not seen in the arteries of the infants. In sclerosis of Mönckeberg's type the calcific deposit is in the media, and the internal elastic lamina acts only as a limiting membrane.

Adult arterial calcification with a histologic appearance closely resembling the calcification of coronary arteries in infants is found in the medium-sized pericapsular arteries of the thyroid gland. This process has been designated "isolierte Verkalkung der Membrana elastica interna"; [23] it may occasionally be seen in arteries in other organs. Only a few of the arteries of a gland may be affected. The deposit is again

in relation to the internal elastic lamina; it may be on both sides or only on the medial side of the membrane; the internal elastic lamina may be continuous through the deposit or may be interrupted; and when calcification is marked the masses occupy the media, with atrophy and replacement of the medial fibers (Fig. 13). Thus it is comparable to the process described in this report. Fibroblastic proliferation of the intima also may occur. These lesions are frequently seen in young persons.

8. Rôle of Elastic Tissue. The rôle of elastic tissue in the pathogenesis of calcific lesions in arterial walls may be important. Wells [24] noted that "elastic tissue seems prone to an early calcification, and it is not uncommon to see the elastic laminae of small arteries calcified in an apparently selective manner." Elastic fibers have been observed to be susceptible to easy damage by toxins, drugs, and other agents.[22] Such exogenous factors may not be necessary. It is unfortunate that no post-mortem examination was made of the sibling of the patient described as case 1, who was reported by the family physician to have died with a similar clinical picture. The primary etiologic feature in all these cases may be congenital weakness of the elastic tissue of the arterial walls. Such a weakness has been postulated as a main factor in the development of arteriosclerosis of the adult type.[24] Chronic illness of the mother might be a cause of this weakness in the child; thus syphilis, allergic diseases, bronchiectasis, and other processes described in the mothers of the various infants might exert a common effect on the offspring. When calcification of this type has occurred in infants in arteries other than coronary arteries, various maternal diseases have been reported by which the elastic tissue could be similarly affected. Acting on such an imperfect ground substance, a variety of factors may cause additional degeneration and subsequent calcification. Although renal disease in some children is associated with arterial calcification, this is not true of all. Similarly, not all patients with hyperparathyroidism nor all with primary bone disease show vascular lesions. The dose of vitamin D which is toxic and which may lead to degenerative lesions may vary with the constitution of the individual. Lippincott [25] has reported a case of possible hypervitaminosis D in an infant, 10 months old; a twin brother (with probable similar biologic characteristics) exhibited symptomatology to a less degree. When, as in experimental animals, dosages beyond the general toxic levels are given, even the best tissue may be affected. Likewise, other dietary factors, infection, or any other extrinsic or intrinsic factor may be the precipitating mechanism which can act upon the embryonally weak protoplasm. This possibility as a cause of the calcification, although the

most attractive, requires experimental demonstration for complete acceptance.

Summary

Five cases of calcification of the wall of the coronary arteries of infants, with associated fibroblastic proliferation of the intima, have been described. The calcification occurs in relation to the internal elastic lamina. In one case the lesion was the probable cause of death; in three others it may have been a contributing factor. Various possible etiologic factors have been discussed. It is considered most probable that no single etiologic factor is present other than an embryonally defective elastic tissue in the arterial walls, upon which various factors can act to cause calcification. Such calcification with associated fibroblastic proliferation of the intima is the most frequent cause of coronary occlusion in infants.

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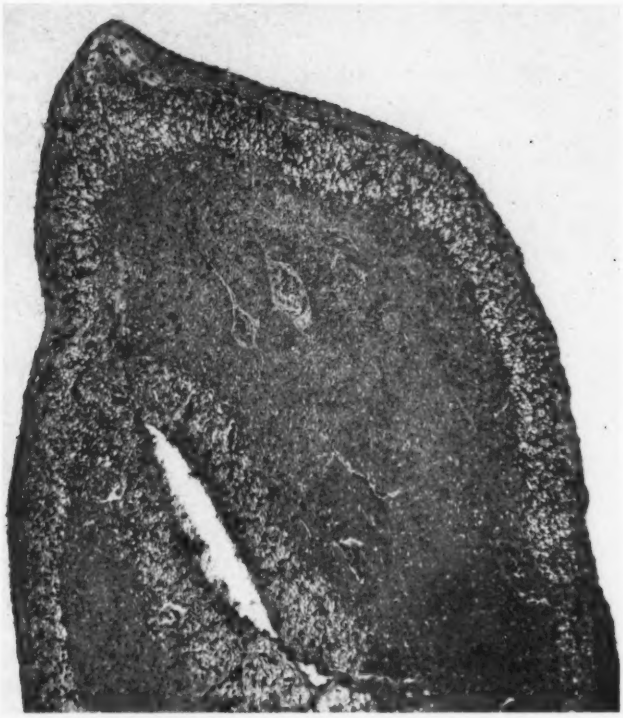
DESCRIPTION OF PLATES

PLATE 191

FIG. 1. Case 1. (Age, 3 months.) Infarction of the central portion of a papillary muscle. Hematoxylin and eosin stain. × 100.

FIG. 2. Case 1. Coronary artery showing calcification of the internal elastic lamina and adjacent inner media. Fibroblastic proliferation of the intima with newly formed blood-channels. Hematoxylin and eosin stain. × 150.

1



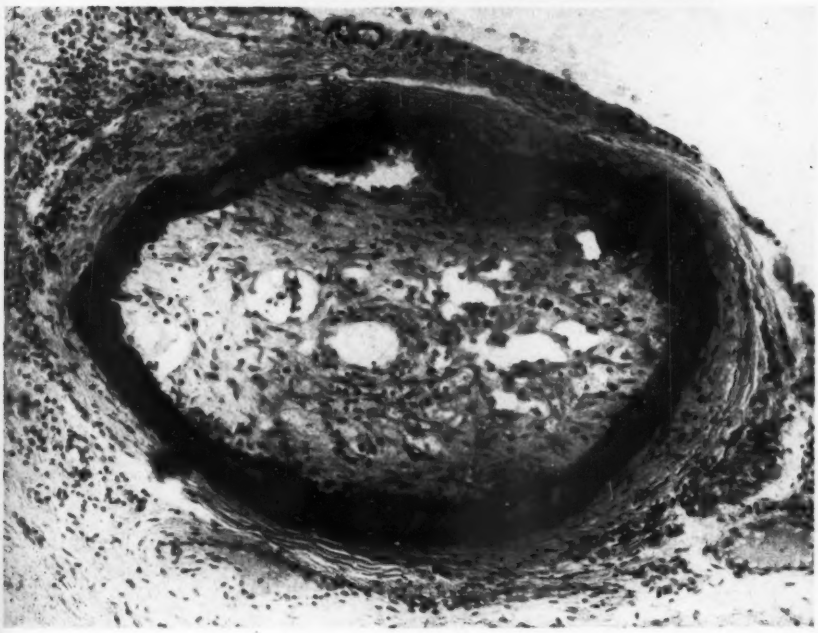


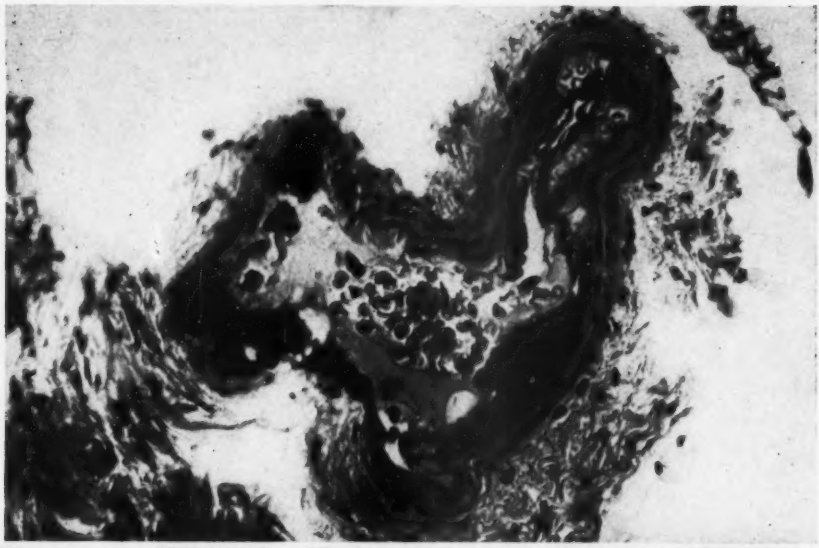
Plate 192

Fig. 3. Case 1. (Age, 3 months.) Focal calcification of the internal elastic lamina and adjacent tissues on both sides in a small artery at the hilum of a kidney. Hematoxylin and eosin stain. × 350.

Fig. 4. Case 2. (Age, 2 months.) Localized calcification of the wall of the splenic artery. (The section was focused so as to emphasize the internal elastic lamina.) Hematoxylin and eosin stain. × 60.

Fig. 5. Case 2. Higher magnification of a localized area of calcification in the same splenic artery. The internal elastic lamina can be identified in the midst of the calcium. Hematoxylin and eosin stain. × 230.

3



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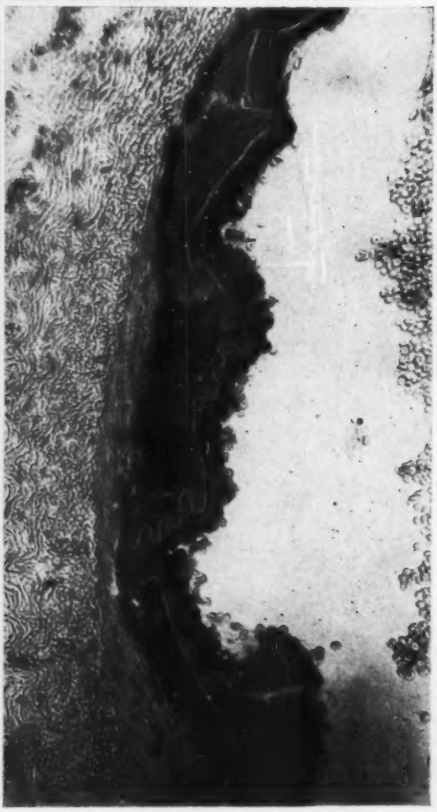


5

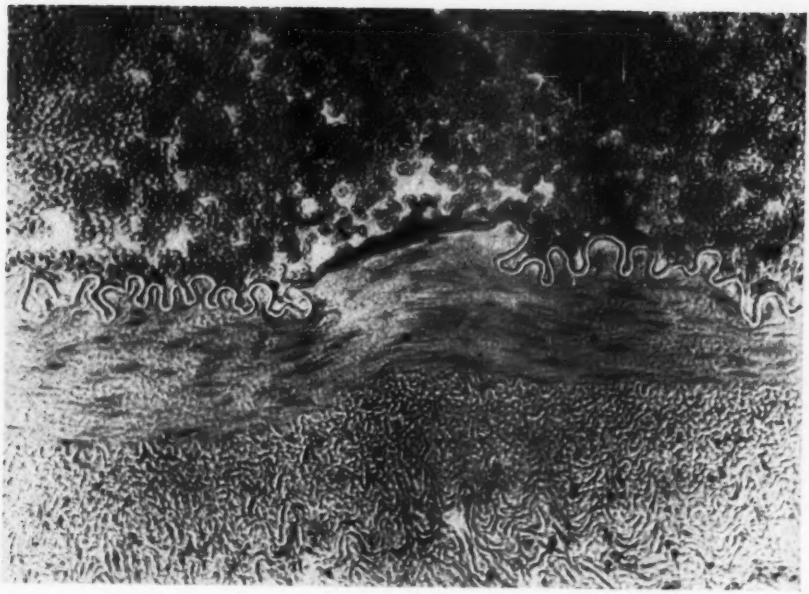
Stryker

Coronary Arterial Calcification in Infancy

PLATE 193

FIG. 6. Case 2. (Age, 2 months.) Higher magnification of another portion of the splenic artery used for Figures 4 and 5, showing deposition of calcium on the inner side of the internal elastic lamina. (The section was focused so as to emphasize the internal elastic lamina.) Hematoxylin and eosin stain. × 225.

FIG. 7. Case 3. (Age, 6 months.) Coronary artery showing calcification along the course of the internal elastic lamina. Focal interruption of continuity of the lamina. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain. × 50.



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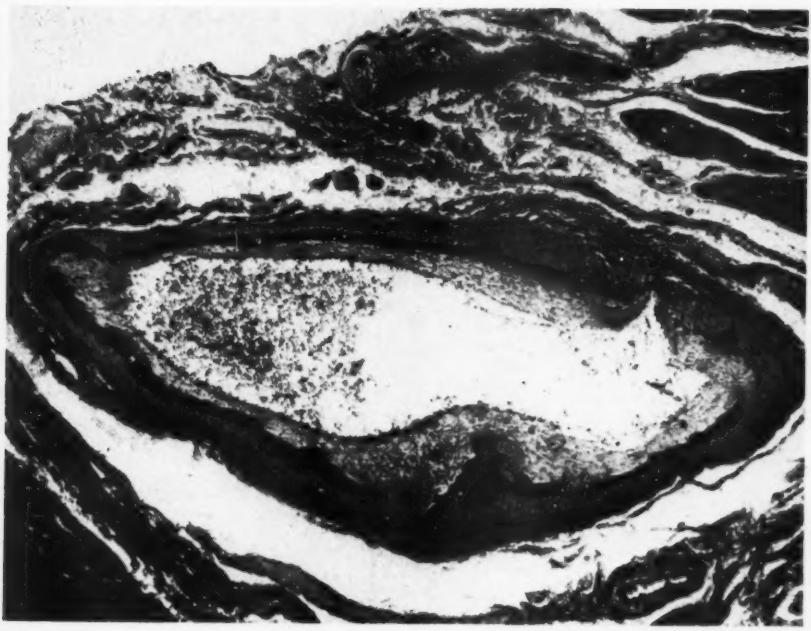

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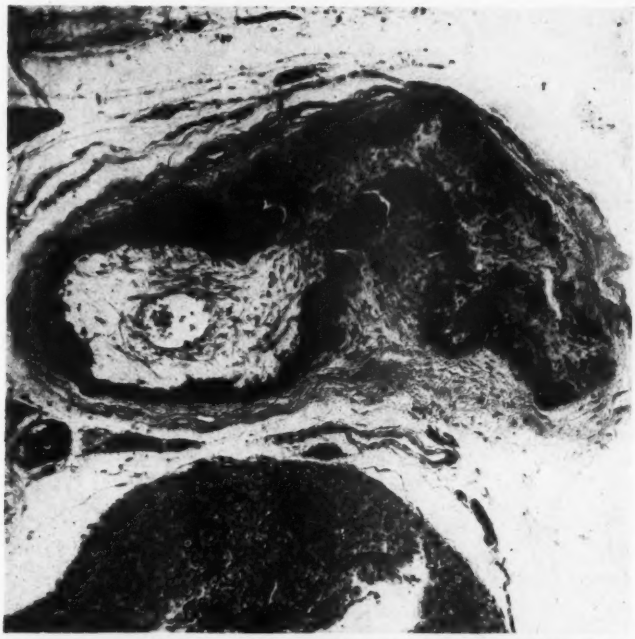
PLATE 194

FIG. 8. Case 3. (Age, 6 months.) Artery in periadrenal connective tissue showing marked calcification in the region of the internal elastic lamina; the lamina cannot be identified. Marked fibroblastic proliferation of the intima with nearly complete occlusion of the lumen. Hematoxylin and eosin stain. × 100.

FIG. 9. Case 4. (Stillborn.) Focal calcification on both sides of elastic fibers in the pulmonary artery. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain. × 30.

8



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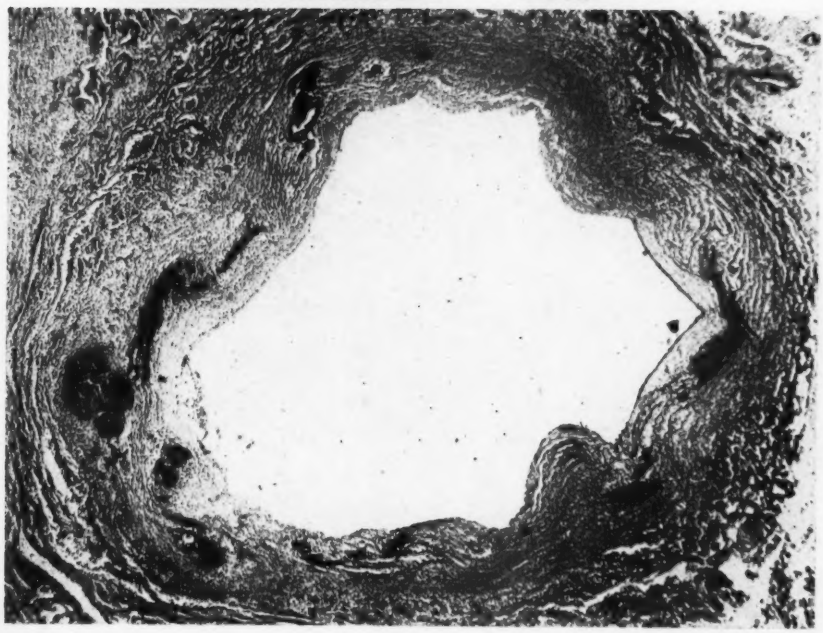


PLATE 195

FIG. 10. Case 5. (Age, 7 months.) Coronary artery with calcification of the internal elastic lamina and of the tissues on both sides of it. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain. × 100.

FIG. 11. Case 5. Marked calcification of the wall of a pancreatic artery. Fibroblastic proliferation of the intima in the region of calcification. Hematoxylin and eosin stain. × 100.

10

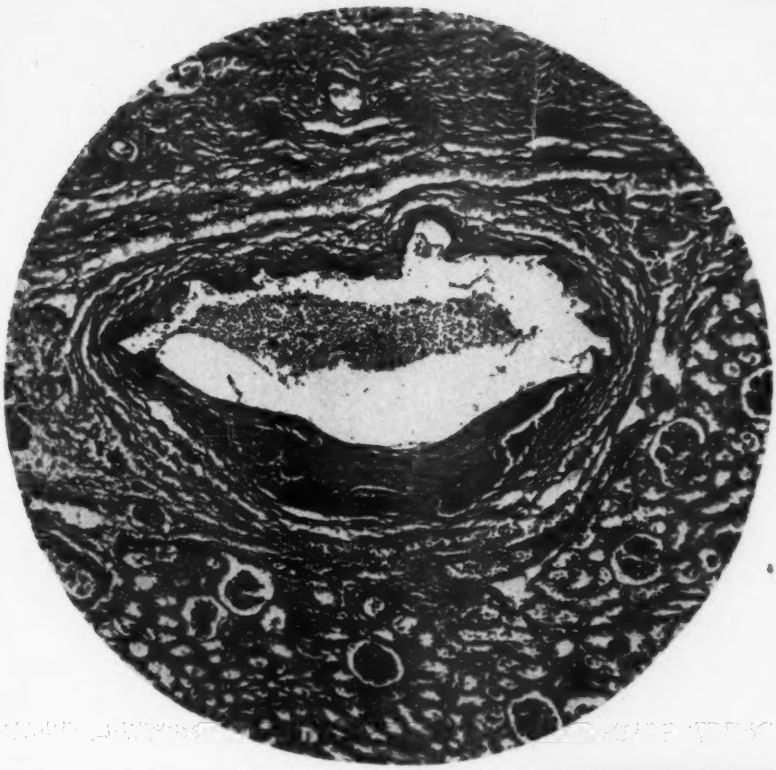
11

PLATE 196

FIG. 12. Case 5. (Age, 7 months.) Focal calcification along the course of the internal elastic lamina of a renal artery. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain. × 100.

FIG. 13. (Middle-aged adult.) Calcification of the internal elastic lamina of a pericapsular artery of the thyroid gland. Small deposits of calcium on both sides of the lamina. Fibroblastic proliferation of the intima. Hematoxylin and eosin stain. × 30.

12



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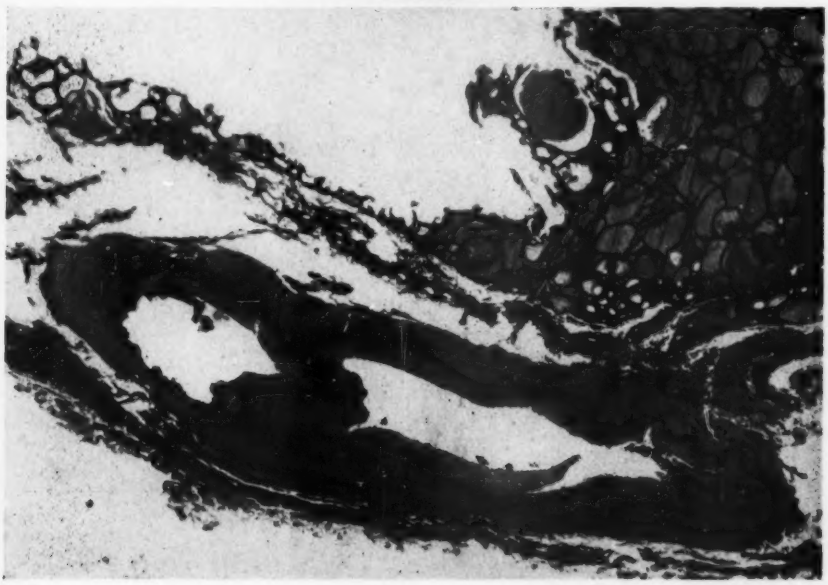

SKELETAL CHANGES CAUSED BY THE COMBINED ADMINISTRATION OF THYROXIN AND ESTROGEN *

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In growing animals, both estrogenic and thyroid hormones accelerated skeletal ageing.[1,2] However, the two hormones exerted different effects on the three phases of the skeletal time curve.[1,3] Thyroxin promoted only to a minor degree the first phase, that of growth; it hastened the onset and progress of the second phase during which degeneration of cartilage is predominant; it stimulated, in particular, the third phase during which resorption of cartilage and bone leads to epiphyseo-diaphyseal union. Estrogen, on the other hand, suppressed growth, accentuated degeneration, and markedly inhibited resorptive processes. When applied simultaneously, the two hormones might neutralize each other, or a combination effect might ensue as was seen after combined administration of anterior hypophyseal and estrogenic hormone.[4] Under the latter conditions, each hormone tended to exert its own action on cartilage and bone: The growth inhibition caused by estrogen, when given singly, was decreased, but there was an intensification of the ageing of cartilage and of ossification over that caused by either hormone alone. The present investigation was undertaken in order to study a possible modification of the estrogen effect by thyroxin.

MATERIAL AND METHODS

Sixty-three virgin mice of the closely inbred strain C57 black, 5 to 6 weeks old, kept on a standard diet of Purina Dog Chow and water, were used in these experiments. The mice were divided into the following five groups: Group I: Twenty-six animals were injected subcutaneously with 100 rat units of alpha estradiol benzoate in sesame oil once a week and with 0.1 mg. of thyroxin three times a week.† Four mice each were thus treated for 1, 2, 4, or 8 weeks, and 2 animals each for 4, 8, 10, 12, or 14 months. Group II: Nine mice received estrogen only for periods corresponding to those in Group I. Group III: Nine animals were injected with thyroxin alone. Group IV: Nine mice remained as untreated controls. Group V: Ten animals were given 200 rat units of the estrogen once a week for 4 weeks. Thereafter the estrogen treatment was discontinued and 5 of these mice were injected

* Aided by the Louis M. Monheimer Memorial Fund.
Received for publication, September 17, 1945.

† We are indebted to Dr. Erwin Schwenk of the Schering Corporation for a generous supply of Progynon-B and thyroxin.

with 0.1 mg. of thyroxin three times weekly for 1, 2, 4, 6, or 8 weeks. At the end of each period, one mouse was sacrificed together with one control that had not received thyroxin.

In all animals the lower end of the femur and the upper end of the tibia were removed as a whole, and sections prepared for histological studies.

Histological Examination

A. Epiphyseal Disk

Under the combined influence of estrogen and thyroxin, the age changes in the growth zones at any given stage were markedly advanced over those found in untreated animals. After 1 or 2 weeks of treatment, the epiphyseal disks were narrower and more heavily calcified than ordinarily. Although the columnar and hypertrophic cartilage cells were only slightly less numerous than usual, they were distinctly smaller. Thus the cartilage cell rows were shorter than in untreated mice of the same age. Under the influence of estrogen alone, the cartilage cells were not only markedly decreased in size but also in number. On the other hand, after injections of thyroxin only, the conversion of the proliferating columnar cartilage into hypertrophic cartilage was intensified.

These changes were even more pronounced when the hormones were allowed to act for 8 weeks. In Figures 1 to 4, the growth zones of 3-months-old untreated and treated mice are shown. In the control animal (Fig. 1), the individual cartilage cell rows were regularly arranged, separated from one another by thin layers of ground substance and composed of eight columnar and two or three hypertrophic cartilage cells. In the animals receiving both hormones (Fig. 2), the matrix was more abundant and hyalinized, the cell rows were scarcely half as high as ordinarily, calcification as well as destruction of cartilage was marked, and amorphous plugs of disintegrated or ossified cartilage cell rows were noted. In untreated mice, a comparable condition was present at about the age of 6 months. In animals injected for the same length of time with corresponding doses of estrogen only (Fig. 3), calcification of the epiphyseal cartilage was accentuated and the proliferation and hypertrophy of the cartilage cells were decreased to a greater extent. Under the influence of thyroxin alone (Fig. 4), resorption of the cartilage was more marked than after the combined administration of both hormones.

In prolonged experiments, the differences between control and experimental animals became still more conspicuous. In Figures 5 to 8, the findings in 7½-months-old control mice and in animals treated for 6 months are demonstrated. In untreated mice (Fig. 5), the growth

zone still consisted of a continuous layer of calcified cartilage containing thick amorphous plugs of destroyed cartilage. Under the influence of both hormones (Fig. 6), the zones of endochondral ossification had been converted into a thin bony scar showing some remnants of calcified cartilage. In noninjected animals, a similar condition was not observed before the end of the second year of life. In mice receiving only estrogen (Fig. 7), the epiphyseal disk was composed of a layer of densely calcified, inactive cartilage showing little resorption. After treatment with thyroxin alone (Fig. 8), a thin, osseous lamella had taken the place of the former growth zone, and there were wide communications between epiphysis and diaphysis. The resorptive processes thus surpassed those noted under the combined influence of both hormones.

Simultaneous treatment with both hormones for 12 months and more led to complete epiphyseo-diaphyseal union. Such an advanced degree of skeletal development was observed likewise after injections of thyroxin alone, but not in untreated old mice nor in those receiving estrogen only.

B. Diaphysis

In mice treated with estrogen and thyroxin simultaneously for 1 or 2 weeks, the metaphysis contained more fibrous tissue and fewer capillaries than ordinarily. There was little replacement of the hypertrophic cartilage by bone; the osseous spicules were broader, firmer, and longer than usual, and here and there they were linked with one another and the shaft by bony bridges. The shafts were thickened and the vascular canals narrowed; the periosteal and endosteal connective tissue was dense and contained abundant collagenous fibers.

In noninjected mice 3 months of age, the replacement of cartilage by bone was progressing actively (Fig. 1). In animals of corresponding age treated with both hormones for 2 months (Fig. 2), a transverse bony lamella, here and there corroded by advancing capillaries, delimited the epiphyseal cartilage from the metaphysis. The spicules were thick but in some places resorbed by bone marrow; the interlacing of the trabeculae was less conspicuous than previously. The vessels of the shaft were enlarged and the periosteal and endosteal connective tissue was loosened. Under the influence of estrogen alone (Fig. 3), thick interlacing bone filled the proximal part of the marrow cavity. The osseous shaft was heavily calcified and showed narrowed vessels; the periosteal and endosteal connective tissue was denser than usual and poorly vascularized. In animals receiving only thyroxin (Fig. 4), the primary trabeculae were resorbed; the bone marrow was rich in capillaries, and the vessels perforating the shafts were enlarged.

After 6 months of combined treatment with both hormones, much of the excessive bone had been dissolved; the diaphysis again contained hemopoietic marrow (Fig. 6), and only fragments of small spicules. The compacta of the shaft was thin and showed numerous congested vessels. After injections of estrogen alone (Fig. 7), resorption of bone, although indicated by the formation of fibrous tissue along the trabecular network, was by far less active than after the simultaneous injections of both hormones. By contrast, in animals receiving only thyroxin (Fig. 8), the diaphysis was cleared of all primary bone; the marrow contained enlarged capillaries; the shafts were thinner than ordinarily; the vessels perforating the shaft were enlarged; and the periosteal connective tissue was loose.

In mice injected for 12 months and more with both hormones, all trabecular bone had been removed and the shafts were thinner than in untreated animals. The conditions thus resembled those seen subsequent to the administration of thyroxin alone for the same period, whereas in animals treated with estrogen only, the metaphysis still was delimited from the epiphysis by a bony plate. Moreover, strands of fibrous tissue indicated that hyperossification had taken place at some earlier date.

CHANGES CAUSED BY ESTROGEN AND SUBSEQUENT ADMINISTRATION OF THYROXIN

When thyroxin was given following treatment with estrogen for 4 weeks, the age changes in the cartilage, already accelerated and intensified by the estrogen injections, were further increased. Plugs of disintegrated cartilage cells appeared in the greatly narrowed growth zones. After 8 weeks of treatment with thyroxin, the resorptive processes were so pronounced as to produce perforations of the epiphyseal plates.

In order to evaluate the part played by thyroxin in these effects, they were compared with conditions in mice that had received estrogen for 4 weeks, but no further treatment. In these animals, proliferation and hypertrophy of the epiphyseal cartilage were resumed, the growth zones again became wider and the matrix looser. In spite of the resumption of the growth processes, however, the histological age of the epiphyseal cartilage in 4-months-old mice was advanced about 3 months over that seen in untreated animals. On the other hand, the epiphyseal plate of the corresponding mouse treated in addition with thyroxin resembled that of an untreated animal toward the end of the first year of life.

In the metaphysis, thyroxin given subsequent to estrogen accelerated the resorptive processes as compared with those in the mice receiving

no thyroxin; 4 and 8 weeks after discontinuation of the estrogen injections there was still a thick layer of subepiphyseal bone and of thick and dense spicules in the marrow cavity; however, an interlacing osseous network was no longer noticeable. Under the additional influence of thyroxin, a good deal of the excessive bone had disappeared after 4 weeks of treatment, and after 8 weeks the subepiphyseal bony plate was reduced to a thin lamella and the marrow cavity was cleared of all trabecular bone. The intensified resorption was also indicated by the more frequent appearance of multinucleated giant cells and by increased vascularization of the connective tissue and the shafts.

Discussion

The findings in the skeleton of (a) untreated mice, and the changes produced by (b) estrogen, (c) thyroxin, and (d) the combined administration of these two hormones are schematically presented in Tables I and II.

Table I summarizes the findings after 2 months of observation. The combined action of thyroxin and estrogen manifested itself as follows: The addition of thyroxin did not materially affect the decrease in the proliferation of the cartilage caused by estrogen; however, it partly counteracted the marked inhibition of hypertrophy and resorption of the cartilage called forth by estrogen. Under the combined influence of these hormones, degeneration of cartilage was intense; on the other hand, the metaphyseal connective tissue was less hyalinized, and the bone present was less abundant and more loosely knit than after injections of estrogen only.

Table II demonstrates the findings after 6 months of observation. When both hormones were given, ossification and particularly resorption of the epiphyseal cartilage resembled closely the same processes seen after administration of thyroxin alone; they were advanced over the normal, although less so than under the sole influence of thyroxin. Likewise, the resorption of bone in the shaft and metaphysis was more conspicuous after administration of both hormones than after that of estrogen alone.

Correspondingly, when thyroxin was allowed to act subsequent to the treatment with estrogen, resorption was resumed more rapidly and it proceeded with greater intensity than in animals receiving no further injections. However, this reversal of the estrogen effect concerned primarily the changes in the bone; in the cartilage, age changes due to thyroxin were added to those caused previously by the estrogen.

When administered simultaneously, the two hormones competed with each other in enforcing their characteristic effects on the skeletal time curve. Each hormone predominated during that phase in which

TABLE I

SCHEMATIC PRESENTATION OF SKELETAL FINDINGS IN NORMAL AND EXPERIMENTAL MICE AFTER 2 MONTHS OF OBSERVATION

		(a) *Untreated*	(b) *Estrogen*	(c) *Thyroxin*	(d) *Estrogen and thyroxin*
Epiphyseal cartilage	Proliferation:	in progress	decreased	ceased	decreased
	Hypertrophy:	in progress	decreased	ceased	somewhat decreased
	Degeneration:	absent	marked	marked	marked
	Resorption:	in progress	greatly decreased	increased	somewhat decreased
Metaphysis	Connective tissue:	loose, vascular	hyalinized; vascularization greatly decreased	vascularization increased	vascularization and hyalinization decreased
	Bone:	thin trabeculae	resorption greatly decreased; interlacing thick bone	trabecular bone resorbed	resorption somewhat decreased; moderately thick trabeculae

TABLE II

SCHEMATIC PRESENTATION OF SKELETAL FINDINGS IN NORMAL AND EXPERIMENTAL MICE AFTER 6 MONTHS OF OBSERVATION

	(a) *Untreated*	(b) *Estrogen*	(c) *Thyroxin*	(d) *Estrogen and thyroxin*
Epiphyseal cartilage	Degeneration; resorption and ossification in progress	inactive; calcific; little resorption	resorbed; epiphyseodiaphyseal union	scanty remnants of inactive cartilage in thin bony scar
Metaphysis	Hemopoietic marrow; trabeculae resorbed	some interlacing of trabeculae	hemopoietic marrow; trabeculae resorbed	hemopoietic marrow; remnants of bony spicules
Shaft	Moderately thick	thickened; vascularization decreased	thinned; vascularization increased	thin; vascular

it acted most conspicuously when injected singly. During the first phase the inhibition of proliferation of cartilage caused by estrogen was not counteracted by thyroxin; however, there was an effective antagonism on the part of thyroxin as to the hypertrophy of cartilage; during the second phase, thyroxin did not diminish the degeneration nor the calcification of the cartilage called forth by estrogen; during the third phase, the influence of thyroxin became predominant, and the aged cartilage responded readily to the resorptive action of thyroid hormone.

Thus, in spite of their antagonistic activities, thyroxin and estrogen did not neutralize each other in respect to their skeletal effects in growing mice. On the other hand, in chickens [5] and pigeons [6] receiving equal amounts of the two hormones, the rise of serum calcium, phosphorus, and cholesterol occurring under the influence of estrogen was prevented; but in pigeons [6] thyroxin given at this ratio did not measurably modify the medullary bone formation called forth by estrogen. Our mice received six times as much thyroxin as estrogen. It is possible that in other species also, the skeletal effects of estrogen might be modified by increasing the dose of thyroxin.

Until more accurate data are established concerning the effects of thyroxin and estrogen respectively on local tissue metabolism and vascular activity, no definite correlation seems possible between such effects and the morphological changes in the skeleton. The scanty and contradictory data, however, suggest that such correlations do exist.[7] Histologically, the antagonism of these two hormones is more conspicuous in tissue near vessels, such as the metaphysis and shaft; it is less obvious or even absent in the nonvascularized cartilage. Enlarged capillaries—a thyroxin effect—have greater resorptive power due perhaps to an increased permeability of their walls. Conversely, a coat of unyielding hyaline material as well as of fibrous tissue [1,8]—an estrogen effect—presents an obstacle to the processes of resorption; in addition, it may decrease the ability of the capillaries to dilate. Furthermore, increased vascularization may allow a more active exchange of substances between blood and tissues and thus prevent the accumulation of inert material such as hyalin. However, the two hormones might also affect directly the metabolism of the skeletal tissues. In mice, thyroxin markedly increases the turnover of radioactive phosphorus.[9] Estrogen, on the other hand, decreases, at least temporarily, the water content of heart and muscle, pancreas, and brain.[10] A similar effect on the bone marrow would be quite compatible with the structural changes produced by estrogen. The percentage of water in bone itself is decreased [11] under the influence of estrogen.

Summary

In growing mice, the skeletal effects of estrogen can be modified but not prevented by simultaneous administration of thyroxin. Thyroxin partly restores both hypertrophy of cartilage and resorption of cartilage and bone which are markedly inhibited by estrogen. However, thyroxin does not counteract the suppression of proliferation of the cartilage and its regression caused by estrogen. The interaction of the two hormones results in a combination effect, with estrogen predominating during the earlier, and thyroxin during the later, stages of administration.

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DESCRIPTION OF PLATES

Plate 197

Fig. 1. Growth zone at the upper end of the tibia of an untreated mouse of strain C57, 3 months old. The cartilage columns show regular configuration, the primary spicules are long and delicate. × 170.

Fig. 2. Growth zone at the upper end of the tibia of a mouse of strain C57, 3 months old, which had received 100 rat units of estrogen once and 0.1 mg. of thyroxin three times weekly for 8 weeks. The epiphyseal plate is narrowed, and growth of cartilage is markedly decreased. There is a large osseous plug present in the growth zone. The primary spicules are longer and thicker than in Figure 1, but not as thick and interlaced as in Figure 3. × 170.

1

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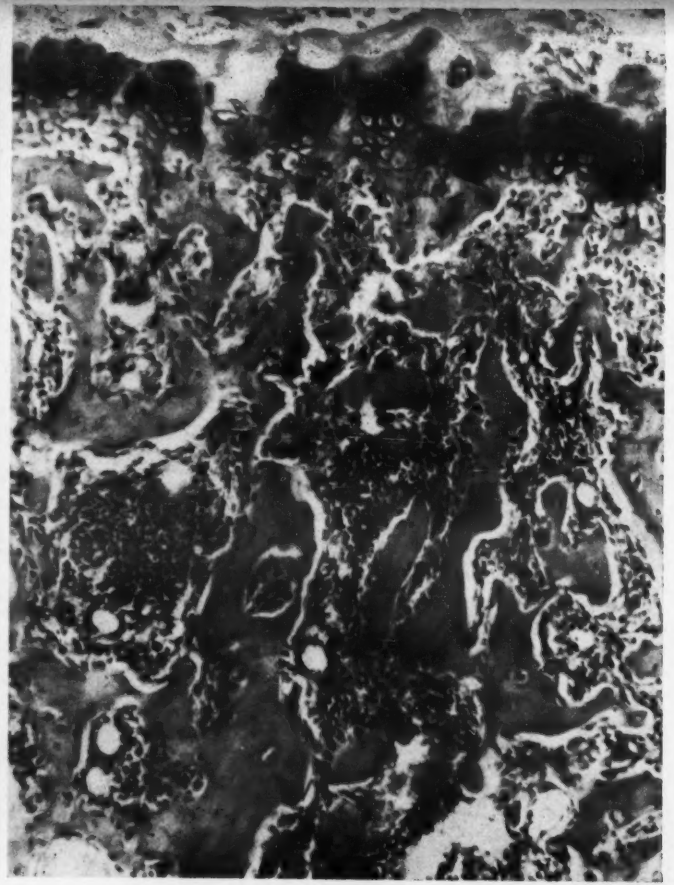

2

Thyroxin and Estrogen

PLATE 198

FIG. 3. Growth zone at the upper end of the tibia of a mouse of strain C57, 3 months old, which had received 100 rat units of estrogen once a week for 8 weeks. The layer of cartilage is somewhat narrower than in Figure 2. There are no hypertrophic cartilage cells present. Calcification and plug formation are of about the same order as in Figure 2. Dense interlacing bone is found in the metaphysis. × 170.

FIG. 4. Growth zone at the upper end of the tibia of a mouse of strain C57, 3 months old, which had received 0.1 mg. of thyroxin three times weekly for 8 weeks. Growth of cartilage has ceased as indicated by a solid osseous plate underneath the degenerated and ossifying cartilage. The primary spicules have been resorbed. × 170.

3

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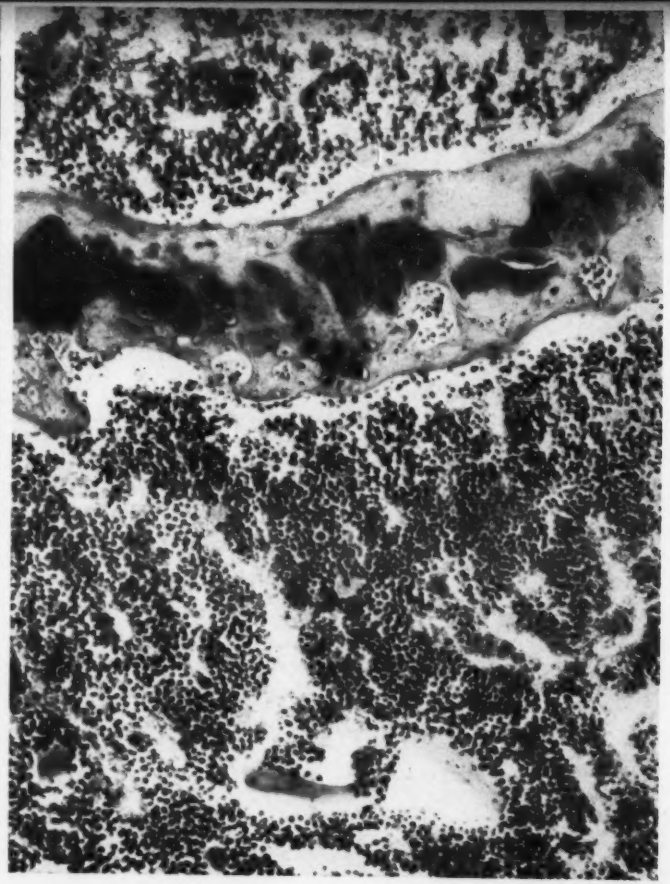


4

Thyroxin and Estrogen

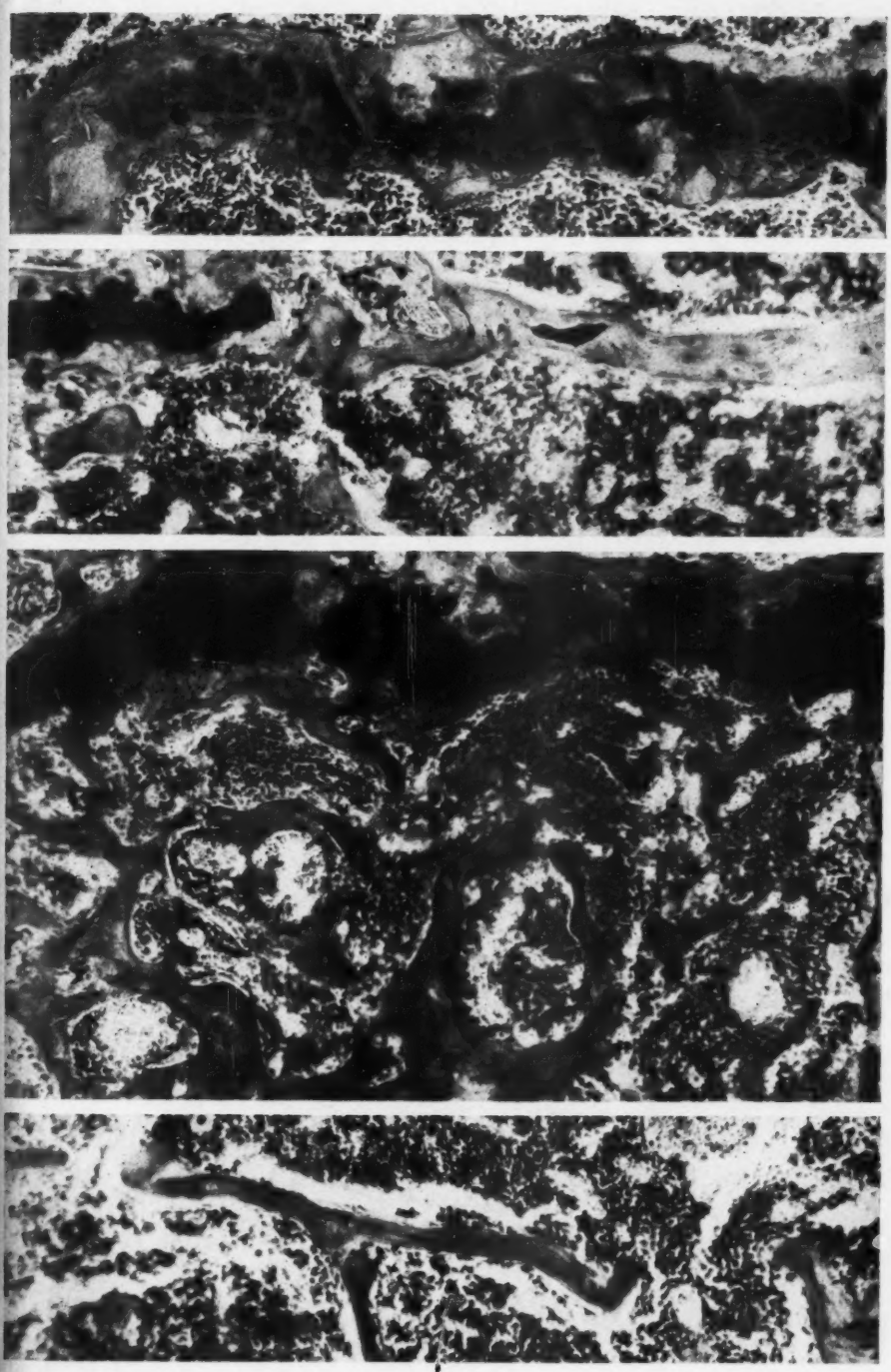
PLATE 199

FIG. 5. Growth zone at the upper end of the tibia of an untreated mouse of strain C57, 7½ months old. Growth of cartilage has ceased. The inactive cartilage still shows a faint columnar arrangement in addition to plugs of degeneration and ossification. Most primary spicules are resorbed. × 170.

FIG. 6. Growth zone at the upper end of the tibia of a mouse of strain C57, 7½ months old, which had received 100 rat units of estrogen once weekly and 0.1 mg. of thyroxin three times weekly for 6 months. The epiphyseal plate still contains some inactive, heavily calcified cartilage, but most of it has been converted into a bony scar. Fragments of bony spicules are present. × 170.

FIG. 7. Growth zone at the upper end of the tibia of a mouse of strain C57, 7½ months old, which had received 100 rat units of estrogen once a week for 6 months. The cartilage is heavily calcified, but shows little ossification and no tendency to perforate. The metaphysis contains a loose network of bony spicules. × 170.

FIG. 8. Growth zone at the upper end of the tibia of a mouse of strain C57, 7½ months old, which had received 0.1 mg. of thyroxin three times weekly for 6 months. There is advanced epiphyseo-diaphyseal union, only thin osseous bars remaining of the former growth zone. × 170.



Silberberg and Silberberg Thyroxin and Estrogen

LEIOMYOMA OF THE VENTRAL LIGAMENT OF THE OVIDUCT OF THE CHICKEN *

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During the course of studies on avian lymphomatosis at this Laboratory, several neoplasms other than those associated with the avian-leukosis complex [1] have been encountered. Of these, the leiomyoma has been most frequently observed, a finding which is in close agreement with that of other investigators. The myoma of smooth muscle has been identified in the chicken and described by several writers.[2-9]

This report will discuss the leiomyoma as it occurred in the 1943 hatched population of the breeding flock of pedigreed White Leghorns at this Laboratory. The apparent close association of the leiomyoma with the normal physiological process of egg production, together with its lack of interference with that function, suggest that the tumor described here will be of interest primarily to oncologists.

Both Jackson [5] and Goss [6] found leiomyoma to be the third most common tumor of chickens, being exceeded by leukotic tumors and adenocarcinomas. Olson and Bullis [7] found leiomyoma to be second in frequency to lymphocytoma. Goss found, in a survey of over 24,000 chickens, 20 cases of leiomyomas in a total of 1,445 birds which had neoplasms. Curtis [8] observed 8 tumors in the oviductal ligament in 880 birds, although she did not identify the tumors histologically.

This tumor had been identified in earlier populations at this Laboratory, but the incidence had been insignificant as compared with that in the 1943 hatched population.

The leiomyoma, according to Boyd [10] and Ewing,[11] is most frequently located in the reproductive organs, particularly in the uterus, although it may occur in other locations which contain involuntary muscle. Feldman [4] stated that in domestic animals the tumor may occur in the uterus, vagina, stomach, alimentary canal, or urinary bladder but is found also in association with the blood vessels, cervix, prostate gland, gallbladder, or spleen. According to Formad,[12] the tumor may also originate in the skin. The location in the chicken is similar to that in mammals in that the reproductive apparatus is the most frequent site.

Olson and Bullis [7] speculated on the causal relationship to egg production but stated that other factors must be involved since all hens with a record of prolonged, heavy production did not show increased incidence of leiomyoma.

* Received for publication, October 2, 1945.

At this Laboratory attention was directed to a further investigation when 291 hens killed at 600 days of age revealed a leiomyoma incidence of 14.8 per cent. Of the 1943 breeding flock of 1,108 females, 59 birds showed the tumor at necropsy, a population incidence of 5.3 per cent. The detection of the tumor was incidental to necropsy of the birds that died during the course of other experiments, or were killed at the termination of the experiment. Since there was continuous mortality in this flock from various causes, only a part of the flock reached productive age. In 703 hens which had laid two or more eggs, the leiomyoma incidence was 8.4 per cent. This group included many birds which died prior to completing a year of production (at about 600 days of age) as well as many others that laid inconsistently. Restricting the data to hens which had completed a year of production, 43 of the 291 were positive for the tumor when examined at 600 days of age. All of these hens had produced eggs, and in all but 3 the organs were in a state of production at necropsy.

Description of the Flock

The 1943 flock of pedigreed Single Comb White Leghorns consisted of 15 lines.* The birds of each line were the progeny of one sire and several dams, the latter being sisters or half-sisters. Thus within each line the hens under consideration were closely related. Each line consisted of from 4 to 8 families † with the exception of one line which contained 17 families. A total of 99 families had an average of slightly more than 11 females per family.

Except from one line, no apparently healthy birds were killed for necropsy prior to approximately 600 days of age. Mortality from lymphomatosis as well as from other causes occurred continuously, however, a factor which would tend to mask the propensity toward this tumor, particularly in those families which had a high mortality prior to heavy production. Two hundred and ninety-one hens were killed at 600 days of age, at which time they had completed 1 year of production.

Description of the Tumor

Gross Appearance. Without exception the tumor was located in the ventral border of the ventral ligament of the oviduct about midway in its length. The ligament is enclosed by the peritoneum, the entire structure being referred to as the mesosalpinx. According to Curtis,[14] the thickened portion of the truncated apex contains smooth muscle fibers, some of which extend radially to, and encircle, the oviduct,

* The term "line" refers to a group of birds with a relatively high *inter se* relationship between sibs or near relatives resulting from restricted ancestry.[13]

† "Family" refers to the progeny of one male and one female.

anastomosing with the muscle fibers of the wall. The tumor developed at the point of convergence of the blood vessels in the mesosalpinx which supply the oviduct. The various other locations described in the literature were not found to be tumor sites in the examples identified.

The 59 leiomyomas of this population ranged from 3 to 40 mm. in diameter. They were irregularly spherical, or were somewhat elongated, particularly those of small dimensions. The tumors were pink to white and some of the larger specimens were marked by large convoluted vessels below the capsule. There was little blood present in the body of the neoplasm, probably due to the density of the structure. The capsule was firm and everted only slightly on section. The tumor itself was firm, dense, and resistant to incision. In the small tumors the consistency was firm but doughy under pressure. The cut surface showed fibrous appearing septa, irregularly placed. These areas had the characteristic sheen of fibrous tissue.

The tumor appeared to have developed symmetrically from the thickest part of the ligament. There was no peduncle formation. The gross appearance was that of a well encapsulated, compact tumor (Fig. 1). It had not reached sufficient size in any case to have interfered mechanically with the passage of the egg through the oviduct, nor with egg expulsion. There was no evidence of metastasis.

Microscopical Appearance. Specimens from 4 cases were fixed in Petrunkevitch's cupric paranitrophenol [15] and sections were stained with hematoxylin and triosin or by Masson's connective tissue method. Three of the cases were considered representative specimens and had shown gross characteristics typical of the majority. These tumors were 4, 7, and 34 mm. in diameter and in each case the hen was in egg production. The fourth case was from a hen in which the tumor appeared to be undergoing regression.

In general, the histological picture was similar to that described by Ewing [11] and Olson and Bullis.[7] There were interlacing bundles of smooth muscle fibers which ran in various directions, giving a whorled appearance to the whole (Fig. 2). The specimens varied in the amount of stromal connective tissue present. The bundles were separated by fibrous tissue and intercellular fibrils were present. There was no indication of inflammation but some round cells were present in 2 cases. In addition to the main bundles of smooth muscle, there was proliferation of the muscle layer of the vessel walls.

In those tumors which appeared to be undergoing regression, there was a greater proportion of connective tissue, not only in the septa but throughout the areas of muscle tissue.

Incidence and Relations

Lines. The incidence of leiomyoma in the entire population, in hens that had laid, and in those that lived for 600 days, has been given in the introduction. The leiomyoma was present in 10 of the 15 lines of chickens and was not identified in 5 lines. In the 10 lines carrying the neoplasm, the incidence ranged from about 1.5 to 18 per cent. However, when only hens that had laid are considered, the incidence ranged from 2.0 to 27.3 per cent. When the number of hens was further restricted to those that had lived to 600 days (all of which had laid), the incidence was from 3.4 to 50 per cent (Table I). Five lines had

Table I

Incidence of Leiomyoma in 15 Lines Figured on Total Flock, Females That Laid, and Females That Lived to 600 Days

Line	No. of cases of leiomyoma	Total no. of ♀♀	Tumor incidence	Total no. of ♀♀ that laid	Tumor incidence	No. of cases of leiomyoma	No. of ♀♀ that lived to 600 days	Tumor incidence
			(*per cent*)		(*per cent*)			(*per cent*)
1	3	48	6.25	30	10.00	2	16	12.50
2	0	53	0.00	21	0.00	0	7	0.00
3	9	50	18.00	33	27.27	9	18	50.00
4	2	61	3.28	42	4.76	2	21	9.52
5	0	47	0.00	25	0.00	0	19	0.00
6	1	68	1.47	49	2.04	1	29	3.44
7	1	75	1.33	50	2.00	1	29	3.44
8	0	57	0.00	36	0.00	0	13	0.00
9	5	52	9.62	25	20.00	3	10	30.00
10	4	95	4.21	56	7.14	3	39	7.69
11	0	40	0.00	14	0.00	0	2	0.00
12	1	51	1.96	42	2.38	1	14	7.14
13	11	72	15.30	50	22.00	11	31	35.48
14	0	80	0.00	50	0.00	0	11	0.00
15	22	259	8.49	180	12.22	10	32	32.25
Total	59	1,108	5.32	703	8.39	43	291	14.77

no leiomyomas; 5 lines had an incidence of from 2 to 5 per cent, and 3 lines an incidence of about 6 to 10 per cent. The remaining 2 lines ranged from about 15 to 18 per cent. These data suggest that this tumor was more prevalent in hens which had laid over a prolonged period and that there was a marked variation in incidence between lines.

Families. The tumor was present at necropsy in 30 of 99 families. Each of the 10 positive lines contained some families which were negative for this tumor. In other words, in no line was the tumor present in all families of that line. It might appear, *a priori*, from the incidence in certain lines and families, that genetic susceptibility plays the most important part in the occurrence of the leiomyoma. However, genetic influence was apparently masked by other disposing factors of age and egg production as well as the intercurrent disease of lympho-

matosis. Lines and families which were susceptible to lymphomatosis died at an age below that of the heavy production period. Under such circumstances the propensity to this neoplasm would not become manifest.

Age. Birds of the 10 positive lines averaged 415 days of age and those of the 5 lines which were negative averaged 333 days of age at necropsy. All negative lines were lower in average age at necropsy than the average of the positive lines; however, of the 10 positive lines, one line was below the negative line average. The absence of the tumor in the lines which died at an earlier average age suggests that the tumor might have occurred within those lines had the life span equalled that of the positive lines. Disregarding lines or families, birds which showed no evidence of the neoplasm averaged 371 days of age at necropsy,

TABLE II

Number of Hens without Tumors Which Laid More Eggs than Average of Line

Positive lines	Average number of eggs by positive ♀♀	No. of negative ♀♀ which laid more than average of positive ♀♀
1	188	2
3	213	2
4	166	12
6	127	23
7	301	3
9	228	3
10	237	2
12	167	12
13	206	9
15	215	7

while positive birds averaged 591 days of age when examined. There were, however, 236 negative laying hens which were older than 591 days at necropsy. Likewise, there was one hen with leiomyoma under 371 days of age at necropsy. These data illustrate the overlapping of ages in the positive and negative birds, and indicate that age alone is not the most important factor in the occurrence of leiomyoma.

Egg Production. The positive birds which completed a year of production averaged 199 eggs for that year; whereas, laying negative birds averaged 155 eggs for a similar period. In the entire population there were 46 negative hens, 600 days old, which laid more eggs than the average of the positive hens. The production for hens which completed the first year of laying ranged in the positive cases from 126 to 250 eggs while negative laying hens for a similar period ranged from 2 to 281 eggs. All 10 positive lines contained some negative hens which laid more eggs than the average of the positive cases in that line (Table II).

Tumor Size. There was no definite relation between tumor size and the number of eggs laid during a given period. Fifteen hens with tumors 3 to 5 mm. in diameter averaged 195 eggs for the first year of laying and 6 hens with tumors of from 20 to 40 mm. in size averaged 215 eggs for the first year of production. Including hens which did not complete a full year of laying, 22 birds with tumors 3 to 5 mm. in diameter averaged 15 eggs per month for the 6 months prior to necropsy, exactly as did 11 hens which had tumors that were 20 to 40 mm. in diameter. In positive birds which had laid no eggs 30 days prior to necropsy the neoplasm averaged 7.9 mm. in diameter. In positive hens which laid 1 to 10 eggs for this period the tumor averaged 13.1 mm. in size, and in hens which laid more than 20 eggs during the 30 days preceding necropsy the neoplasm averaged 10.8 mm. in diameter. Tumor size plotted against the number of eggs laid for 1, 2, 3, or 6 months prior to necropsy showed no definite correlation. In 4 of the 59 cases showing the leiomyoma, the ovary was not in a laying state at necropsy. One hen (E802D) had laid 194 eggs in the first year of production but had laid no eggs for 6 weeks prior to necropsy. The tumor in this case was 4 mm. in diameter. A second hen (E813K) produced 163 eggs the first 12 months of laying and laid 11 eggs in September. She was killed on October 10th and was found to have a tumor of the mesosalpinx, 8 mm. in diameter. Another hen (E257D) had laid continuously for the 10 months before necropsy, producing a total of 175 eggs for that period. This hen had laid 3 eggs in October and was killed October 10th. The neoplasm was 40 mm. in diameter. The fourth bird (E764W) had a 4 mm. tumor. This hen had laid 167 eggs for the first year of production but no eggs were recorded for her for 3 months prior to necropsy. For 2 hens (E606A and E615X) the record showed that no eggs were laid for 7 and 4 months respectively before necropsy. However, at necropsy the ovaries were either in a state of production or approaching production. It should be stated, also, that there was one hen with tumor as well as several negative birds for which there were no records of eggs. It is probable that these hens were "floor-layers."

Discussion

The incidence of leiomyoma in the 1943 hatched flock at this Laboratory was much higher than has been previously reported.[5-7] The incidence in this flock was likewise much higher (nearly 15 per cent) in the group of 291 birds which were killed at approximately 600 days than in birds (about 2 per cent) which were necropsied at ages below 600 days. However, the latter group contained one line of birds which

were destroyed at ages between 500 to 600 days. In the 1,108 birds, only 5 of the 59 neoplasms were detected in birds that had died; whereas, 54 cases were identified in hens that were killed. Of the negative birds which had died, the ovary was found only infrequently to be in a state of production. In such cases cannibalism was usually the cause of death. In the 5 hens which died and which showed the neoplasm at necropsy, the ovary was either in a state of production or a state of regression from recent laying.

It is probably true that a flock of 300 laying hens, as described here, is seldom available for necropsy examination. The high incidence of leiomyoma in this cross section at 600 days suggests that the tumor must tend to regress after the cessation of laying.

Since the tumor occurs with greater frequency in laying birds and apparently regresses, its detection would ordinarily be largely limited to such birds. The tendency to submit only morbid or obviously diseased birds for necropsy would thus result in a lower necropsy incidence than when laying birds are included.

The fact that the tumor did not appear in 5 lines suggests that a hereditary influence is involved. All of the 5 negative lines contained some individuals which had production records equal to those of the positive birds. In general, however, the negative lines and families died at an earlier average age than did the lines or families in which the neoplasm was present. The families that died at an early age—below that necessary for heavy production—obviously did not show the neoplasm. However, an average age of death for a family equal or over that of positive families did not necessarily mean that the neoplasm would be present. In some families which had only one member with neoplasm, the age and production records of that bird were higher than the family average. The fact that the neoplasm was not found in 5 lines suggests that some genetic influence operates to predispose birds of certain breeding to the tumor, provided the egg production factor is also considered.

The age at which the tumor is most likely to appear could not be determined from the data available although from the foregoing it is apparent that the hen must reach maturity since sustained production was a closely associated factor.

The tumor was found only in hens that had produced eggs and usually in the best layers. One hen, however ($E1101J_3$), laid only 40 eggs over a period of 11 months. This hen had laid from 1 to 5 eggs per month. In this case the leiomyoma was only 5 mm. in diameter. Curtis [8] described the ventral border of the ventral ligament as a "muscular cord," 3 to 6 mm. in diameter. That description would

suggest that such a size should be considered normal. In this report such a size was considered as gross evidence of tumor involvement. The one 4 mm. specimen examined microscopically revealed proliferation of the muscle layer of the vessel walls.

The fact that some hens without tumors had an egg record equal to that of those with tumors within each line suggests that there is, besides heredity and egg production, a third etiologic factor.

There was no relation between tumor size and the number of eggs laid during a given period. However, since neither the time of onset of the neoplasm nor its rate of growth is definitely known, the data given are of questionable value. The fact that the hens with the larger tumors laid only 20 eggs more over a 12-month period than did those with the smaller tumors would suggest that such a slight increase in production would hardly account for the great difference in tumor sizes.

In the flock examined the incidence of this neoplasm was second only to lymphomatosis, which is in agreement with the report by Olson and Bullis.[7] However, in birds killed at 600 days the incidence of leiomyoma was higher than that of lymphomatosis.

The ventral ligament of the oviduct was the only site of leiomyoma in the flock under discussion. This would indicate that the neoplasm is closely associated with the reproductive processes. Other writers have described this neoplasm in several locations in the same bird and have suggested the possibility of metastasis. Jackson [5] stated that all tumors of the smooth muscle cannot be regarded as true leiomyomas, since implantation metastasis from a carcinoma leiomyomatosum may take place with subsequent regression of the epithelial elements in the implant.

Since the neoplasm was observed in only one location in the group examined, that fact as well as the density, encapsulation, and the apparent slow growth of leiomyomas would tend to favor the theory of multiple origin rather than of metastasis when the neoplasm is found in more than one location in the body.

The probability that the tumor is transient, coupled with the fact that hens are frequently not laying when in a morbid condition, paradoxically associates the neoplasm with only laying birds or birds which are normal aside from the tumor. This relationship with normal physiologic activity is unique, especially since the neoplasm does not appear to interfere with the function of laying. Such an association naturally raises the question of whether the leiomyoma is a true neoplasm.

That hypertrophy of the smooth muscle of the ovarian ligament is not necessary for high production is shown by the fact that the de-

velopment referred to as a neoplasm in this report is not consistently found in all high producers. Prolonged functioning of the reproductive organs might conceivably stimulate the unrestricted proliferation of smooth muscle tissue. The pull on the apex of the ligament might thus cause hyperplasia of the smooth muscle fibers. It was noted grossly that the subcapsular vessels were often engorged and convoluted, indicating a local high blood pressure. Such pressure might conceivably be the result of interference with circulation by the contraction of the fibers in the main tumor. If such a process does occur, the increased intravascular pressure might account for the thickening of the vessel walls in that region. Acceptance of this explanation would result in classifying this tumor as a hyperplastic rather than a neoplastic process. Mechanical functional stimulation from continuous production would be indirectly related to a high level of the female sex hormone.

The fact that the tumor does not occur in all birds of equal production records suggests that some unidentified factor is operating to stimulate the muscle tissue to proliferate. In contrast to the above theory of functional stimulation, there may be a direct relationship with the blood level of the female sex hormone. The increase in size of the reproductive organs to accommodate pregnancy in the mammal has its counterpart in the fowl in the hypertrophy of structures associated with egg production. The female sex hormone undoubtedly plays a part in the functional development of the oviduct for egg production and in egg production itself. An extension of the physiological hypertrophy due to a continuous high level of the hormone in the blood is thus conceivable.

While a genetic influence was not definitely identified, the distribution of the occurrence of the tumor suggests that some hereditary relationship does exist.

Summary

1. The leiomyoma of the ventral ligament of the oviduct is of oncological interest because of its close association with normal physiological processes.

2. The relatively high incidence (14.8 per cent) in laying hens killed at 600 days suggests that the tumor may be more common than has been reported heretofore.

3. The gross and histological features indicate that the tumor is benign.

4. The tumor does not appear to interfere with egg production.

5. Theories on the factors related to causation are suggested: (a)

mechanical functional stimulation, (b) increase in the level of the female sex hormone.

6. Since the tumor is not known to be malignant nor to interfere with normal functions, it should be of little concern to the poultry breeder.

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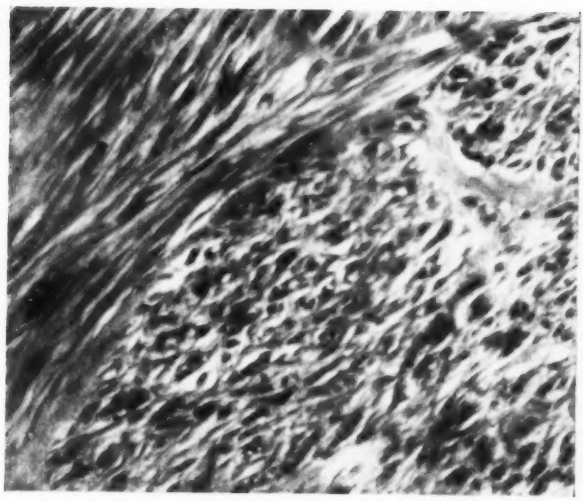
DESCRIPTION OF PLATE

PLATE 200

FIG. 1. Leiomyoma of the ventral ligament of the oviduct of the hen. × ½.

FIG. 2. The histological structure and whorled appearance of the tumor of the ventral oviductal ligament are typical of leiomyoma. × 300.

1



2

THE OCCURRENCE OF NEOPLASMS IN THE LIVER, LUNGS, AND OTHER TISSUES OF RATS AS A RESULT OF PROLONGED CHOLINE DEFICIENCY*

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The influence of diet and separate dietary constituents on the incidence of neoplasms induced by carcinogenic chemicals has been studied by various investigators with conflicting results. So far as we are aware, however, the production of neoplasms as a result of a simple dietary deficiency has not been reported.

Hepatic cirrhosis in animals receiving choline-deficient diets has been produced by several groups of workers.[1-4] An experimental procedure for the consistent production of such a cirrhosis was developed in this laboratory and an intensive study of the pathology involved was undertaken. In the course of the study, the development of various types of neoplasms was observed in what appears to be a significant percentage of the experimental animals. It is the purpose of this paper to describe the sequence of lesions culminating in the appearance of these neoplasms in the liver and other tissues of rats receiving a choline-deficient diet.

Experimental Procedure

In a series of feeding experiments on different diets, it was found that Diet 43A was suitable for producing the lesions described in this paper. Diet 43A consists of 30 per cent alcohol-extracted peanut meal, 6 per cent alcohol-extracted casein, 40 per cent sucrose, 20 per cent pure lard, and 4 per cent salt mixture.† The peanut meal was extracted five times and commercial casein was extracted four times in boiling 95 per cent ethyl alcohol for 2-hour periods. The peanut meal was the usual commercial oil mill product containing 45 per cent of protein. To each kg. of the diet were added 200 mg. of inositol, 10 mg. of calcium pantothenate, 50 mg. of alpha-tocopherol, 4 mg. of riboflavin, 2 mg. of thiamine, 4 mg. of carotene, 2 mg. of pyridoxine, and 0.125 mg. of calciferol.

Rats were started on the experimental diet at the age of 23 days, in individual cages with raised screen bottoms. They received Diet 43A

* Received for publication, October 15, 1945.

Published with the approval of the Director of the Alabama Agricultural Experiment Station.

† Salt mixture 186 modified by the addition of Cu, Zn, and Mn was used. (Guerrant, N. B., and Salmon, W. D. The stability of vitamin G as measured by its growth-stimulating effect. *J. Biol. Chem.*, 1930, 89, 199–211.)

ad libitum. Two rats from each litter were designated as controls and were fed 20 mg. of choline chloride a day for the duration of the experiment. A total of 88 rats were placed on Diet 43A; of this number, 19 were controls receiving choline throughout the experiment. In order to prevent death from acute choline deficiency, it was necessary to feed predetermined minimum supplements of choline to the experimental rats for the first 2 weeks. After this, the dosage was gradually decreased until the experimental animals were deprived of choline entirely. In case of serious weight loss soon after the choline was discontinued, an occasional dose was administered. The aim was to use the minimum dosage necessary to keep the rats alive.

The rats were weighed each day during the first 6 weeks and once a week thereafter. Some were killed for necropsy at regular intervals and their organs preserved for histopathologic studies, so that the sequence of changes occurring in the tissues could be followed; others were kept on the experimental diet until marked symptoms of decline in condition were evident. The animals were killed by decapitation. At necropsy, tissue specimens were taken from each lobe of the liver, and from the spleen, pancreas, lymph glands, lung, kidney, adrenal, thymus, heart, alimentary tract, and any abnormal growth. The tissues were fixed in Bouin's, Zenker-formol, Zenker-acetic, formol, or 70 per cent ethyl alcohol fixatives. Both frozen and paraffin sections were made. The following staining technics were used: hematoxylin and eosin, scarlet red and hematoxylin, Taenzer-Unna orcein and methylene blue, osmic acid, Mallory's triple stain, Nile blue sulfate, Weigert's stain, and Masson's trichrome stain for connective tissue.

Results

Symptomatology

Fourteen (20 per cent) of the rats not receiving choline died of acute choline deficiency during the first 6 weeks on the experimental diet. The remainder of the choline-deficient rats made a moderate growth and remained in reasonably good condition for several months. Following this they gradually became unthrifty in appearance. There were marked loss of hair, muscular weakness, drowsiness, and lethargy. In some, the eyes became lusterless and opaque following earlier hemorrhage. Râles were common in the later stages of the condition.

Early and Transitional Lesions

In animals necropsied during the first 28 days of feeding on the choline-deficient diet, the livers were swollen, abnormally light in color, and increased in weight above the normal. There was a marked ac-

cumulation of fat in the form of globules of various sizes, apparently involving all the liver cells. Some cells were so filled with fat globules that the nucleus was pushed to one side. The staining reactions indicated the presence of both fatty acids and neutral fats.

Necrosis occurred as a sequel to earlier phases of cell degeneration. The first indications of necrosis were distention of the nucleus followed by its shrinkage and condensation into several deeply staining fragments.

Sections from livers of rats that had been on the choline-deficient diet for 3 months showed the peculiar zonal distribution of fatty infiltration and degeneration illustrated in Figure 1. This was designated as a pre-cirrhotic or mild cirrhotic stage. In the fatty zones considerable necrosis was noted and there was a definite increase in fibrous tissue in these regions. In some lobes of the liver, the fibrous tissue completely encircled lobules and groups of lobules; there was also considerable increase in the intralobular connective tissue. Evidences of a rapid rate of cell destruction and cell regeneration began to appear.

Advanced Lesions

All rats that received the choline-deficient diet for 8 months or longer developed advanced hepatic cirrhosis. There was considerable variation in the gross appearance of these livers; some were almost smooth on the surface; others were only slightly roughened or finely granular; the most severe had the characteristic "hobnail" appearance with nodules and pedunculated projections varying from the size of a pinhead to 1 cm. in diameter (Fig. 2). The consistency varied from lobe to lobe. In general, the livers which were least severely affected were largest and lightest in color, indicating the highest fat content. Of the livers examined in this period, 85 per cent were classified as "hobnail" cirrhosis. The typical microscopic appearance of these livers is illustrated in Figure 3. Most of them showed the characteristic perilobular fibrosis with intralobular strands of connective tissue. In most cases the intralobular strands were so prominent as to cause a pseudo-lobulation.

There was considerable variation in the amount of fat in the different lobules of the liver, the fat appearing to decrease as the cirrhosis advanced in extent and severity. While some of the lobules contained considerable fat, others consisted largely of newly regenerated liver cells and contained little or no fat; some were in a state of partial necrosis. This variation is shown in Figure 4.

In 75 per cent of the cirrhotic livers there was a substance distributed in globular form throughout the interlobular connective tissue, and in some cases in the lobules themselves (Fig. 5), that was not dis-

solved by alcohol, dioxane, or xylol. This substance stained red with sudan IV, blue to blue-black with Nile blue sulfate, and black with osmic acid. It is probably identical with the ceroid of Lillie and coworkers,[6] although Diet 43A did not contain cod-liver oil. Its distribution indicated that it is a product of cell degeneration.

The livers of 50 per cent of the animals on the choline-deficient diet contained cysts, which appeared to be agglomerates of dilated sinusoids interrupting the regular architecture of the lobule. There was a focal loss of parenchymal cells, with a proliferation of endothelial cells in these areas and a considerable amount of collagenous fibers between the cyst-like spaces. The cystic spaces in any one area varied in size as shown in Figure 5. These spaces contained a small amount of fibrinopurulent exudate. The cells lining the spaces were in most cases endothelium-like, indicating that they might be derived from the sinusoids. In some cases, however, the spaces were lined by cuboidal or even low columnar cells, indicating that they might be derived from bile ducts. In some lobes of the livers, cells of the endothelial type were so predominant that the regular parenchyma was obscured. The proliferation of endothelial cells with a focal loss of parenchymal cells is illustrated in Figure 6. Areas of this type were very numerous and were found consistently in all the cirrhotic livers.

In some lobes of the livers very numerous mitotic figures were found, indicating that regeneration of new parenchymal cells was taking place at a rapid rate. The portal veins in some of the livers were altered. Their walls were thickened in places by proliferation of connective tissue and the deposition of a substance staining deep blue with hematoxylin. In the most severe cases of cirrhosis of the liver, severe hyperplasia was evident in the lobules and pseudo-lobules as shown in Figure 7. In some lobules there were numerous giant cells arranged in acini and cords resembling a hepatoma (Fig. 8).

In 10 per cent of the animals, there were pedunculated projections from the liver which were larger than the hyperplastic nodules previously described. These neoplasms were usually multiple, varied in size, and appeared to be hepatomas. Some were connected to the liver only by a thin strand of connective tissue, but others were in the mesentery entirely separated from the liver. Some were found in the midst of the hyperplastic liver parenchyma, compressing this tissue. Figure 9 illustrates the distribution of these neoplasms. Histologic preparations of these nodules showed no tendency toward lobulation, a peculiar cell arrangement, and very numerous mitotic figures (Fig. 10). There was very little connective tissue in these areas. As shown in Figure 11, the cytoplasmic membranes were very prominent. The cells varied

in size and, in some parts of the growth, they were arranged in solid sheets, while in others they were arranged in whorls and acini-like structures or in cords of varying thickness (Fig. 12). Some of the cells showed atypical tricentric mitosis. A large percentage of the cells contained from two to four nuclei, and the nucleoli were unusually prominent. Some nuclei were oblong in shape or extremely large and contorted, having one or more indentations. The staining reaction was unlike that of normal liver cells and these areas could be distinguished in the slide because of this difference.

Extensive bile duct proliferation was found in all of the livers of the experimental animals. In most of them these proliferations seemed to follow the connective tissue bands, but in some the growth was so extensive as to involve large numbers of entire lobules and to replace as much as one-third of an entire lobe of the liver. All lobes of the liver were sometimes affected.

The livers of 30 per cent of the 50 rats that survived for 8 months or longer on the choline-deficient diet exhibited neoplasms of the nature of an adenocarcinoma. This growth was entirely different from the numerous areas of proliferating bile ducts; it was characteristically made up of cords of cells, solid masses, and acini-like whorls of stratified cells. The acini-like spaces contained cell débris and fibrinous exudate. The growth differed from the bile duct proliferations in that the epithelial cells of which it was composed were definitely stratified and not in simple acini. There were large numbers of mitotic figures present and there seemed to be little relation between the epithelial cells and the stroma. This type of lesion was not necessarily located at the periphery of the liver and was not represented by a shrunken area, but rather by a massive growth at times compressing liver tissue. As to the origin of the growth, it seemed that in some instances it arose from the liver cells themselves, as can be seen in Figure 13. In some the growth began at the center of the lobule, and in others it seemed to arise in the interlobular spaces, as shown in Figure 14. In still other examples the growth seemed to arise from the cells on the outer margin of the lobule. The growth was so extensive in 25 per cent of the cases that one-third of the lobules of an entire lobe were affected and more than one lobe of the liver was concerned. Figure 15 illustrates the pattern of the growth.

Hemangio-endotheliomas in the peritoneal and subcutaneous fat were found in 10 per cent of the deficient animals. Three of the animals exhibited subcutaneous growths measuring from 1 to 5 cm. in diameter and located in the inguinal region. Two of the animals had growths that were smaller but of the same type located in the peritoneal

fat. Microscopic examination revealed extremely cellular hemangioendotheliomas with large numbers of blood vessels and vascular spaces filled with blood cells. In some cases the structure resembled that of erectile tissue. The cells contained oval or round nuclei with scant chromatin. The cytoplasm was indistinct and stellate or streaming in appearance. Very many mitotic figures were seen in two of the growths but the rest showed only a moderate number. Figure 16 illustrates the structure of this growth.

The lungs of 38 per cent of the animals necropsied after 8 to 10 months on the choline-deficient diet showed nodules varying in size and number, involving various parts and in some cases practically all of the lung. One of the more severely involved lungs is pictured in Figure 17. As a routine procedure, smears were made of the fresh material from these nodules and fixed and stained according to the Ziehl-Neelsen technic. This test showed no acid-fast organisms. Likewise, cultures prepared on various media failed to demonstrate acid-fast organisms. Material from freshly dissected rats was prepared and injected into guinea-pigs. The sites of injection healed without the development of ulcers. After the animals remained healthy for several months they were killed for necropsy. The lymph glands were normal grossly and histologically, as were also the other organs of the body.*

Sections of the pulmonary nodules revealed an abnormal growth consisting of masses of anaplastic oval cells of various sizes. There were numerous acini-like structures of an adenomatous nature, but the larger masses of cells were of the nature of a primary, medullary carcinoma. Figure 18 illustrates the oval or spindle-shaped cells with very prominent oval or round nuclei that stained heavily with hematoxylin. Mitotic figures were very numerous.

The epithelium lining bronchiectatic cavities exhibited marked metaplasia in a number of cases. Figure 19 illustrates a transition of the simple columnar epithelium lining a bronchiectatic cavity to a well differentiated stratified squamous epithelium. Numerous bronchiectatic cavities were present, lined with hyperplastic epithelium which exhibited many mitotic divisions; in most cases there were papillomatous invaginations of epithelial character into these cavities. The papillomatous structures contained sheets of cells which had prominent oval nuclei. The cytoplasmic borders of these cells were ill defined (Fig. 20).

* We wish to express our appreciation to Dr. W. E. Cotton of the School of Veterinary Medicine for assistance in making the tests for acid-fast organisms and the guinea-pig inoculations.

Of the 50 animals, 3 developed retroperitoneal growths, which, through pressure on nerves, produced paralysis of the posterior extremities. The largest of these growths was 3 by 2 by 1 cm., and the smallest was 1 cm. in diameter and rounded. The masses were of firm consistency and were reddish on gross dissection. Microscopic examination revealed neoplasms of the nature of a sarcoma or fibrosarcoma. The amount of connective tissue varied in the different parts of the growths; in some places it was very prominent, but in other parts of the growth it was almost imperceptible or entirely absent. The most characteristic sections of this growth, as shown in Figure 21, were composed of spindle-shaped cells of moderate size, arranged in rows in different planes and closely packed together. In some parts of the growth, connective tissue was more prominent (Fig. 22) but the arrangement of the cells was still characteristically the same. In some places there was infiltration of the muscle layer with destruction and replacement of the normal tissue (Fig. 23). In all of the neoplastic tissue there were very numerous mitotic figures, and in some cases abnormal divisions. Figure 24, which is enlarged to show the nature of the spindle-shaped cells and their nuclei, includes one of these abnormal division figures.

Discussion

The occurrence of neoplasms of various types in 58 per cent of the rats that were kept on a choline-deficient diet for 8 months or longer, while not a single neoplasm developed in litter-mate controls receiving the same diet supplemented with 20 mg. of choline per rat daily, emphasizes the apparent significance of the results.

It appears that marked accumulation of fat in the liver cells resulting from an insufficiency of choline, or more specifically of labile methyl groups, initiates a sequence of responses which result in profound changes in the structure and behavior of the cells. Cell degeneration and cell regeneration are greatly stimulated; connective tissue cells replace normal liver cells; extensive bile duct proliferation, severe hyperplasia, and exaggerated mitotic activity occur; and eventually the abnormal types and patterns of the neoplastic cells appear. The sequence of changes is remarkably similar to that reported in the literature following the administration of carcinogenic hydrocarbons. Interestingly enough, some of the diets used in the study of carcinogenic compounds [6, 7] have been low in methionine and choline. White and Edwards [8] have reported, however, that supplements of methionine and choline do not decrease the incidence of p-dimethylaminoazobenzene-

induced hepatic tumors. On the other hand, Maisin and co-workers, Nakahara, Mori, and Fujiwara, and other investigators are reported by Cook and Kennaway [9] to have shown the presence of anticarcinogenic factors in brain, liver, thymus, bone marrow, yeast, and whole rye flour.

The sequence of changes in the lungs was not followed as closely as in the liver. As previously reported from this laboratory,[10] severe congestion, hemorrhage, and edema in the lung appear in the early stages of choline deficiency. Hydrothorax is a common finding. These early lesions probably are the basis for the development of primary carcinoma in a relatively high percentage of the animals.

Summary

A chronic or prolonged choline deficiency was produced in rats by feeding a diet low in choline and methionine but supplemented by minimum doses of choline to prevent death from acute choline deficiency. Of the 88 rats started, including the controls, 50 survived for 8 to 16 months on the choline-deficient diet. The pathology of chronic choline deficiency in these animals was studied.

Cirrhosis of the liver was consistently found in all of the rats on the deficient diet. A substance resembling "ceroid" was observed in 75 per cent of the cirrhotic livers.

Cysts, characterized by altered sinusoids and bile ducts with a focal loss of parenchymal cells and a proliferation of endothelial cells, were found in the livers of 50 per cent of the animals.

Hepatoma-like neoplasms developed in 10 per cent of the livers. These neoplasms were also found in the mesentery, with a thread-like attachment to the liver in some cases and without any apparent attachment to the liver in others.

Neoplasms of the nature of adenocarcinoma were observed in the livers of 30 per cent of the animals.

Neoplasms that appeared to be primary carcinomas occurred in the lungs of 38 per cent of the rats.

Subcutaneous and peritoneal hemangio-endotheliomas were found in 10 per cent of the animals.

In 6 per cent of the rats, neoplasms that were classified as retroperitoneal sarcomas were found.

Neoplasms of one or more types were observed in 58 per cent of the deficient animals.

No lesions similar to those described were found in the litter-mate controls, receiving the same diet supplemented with 20 mg. of choline chloride per rat daily.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

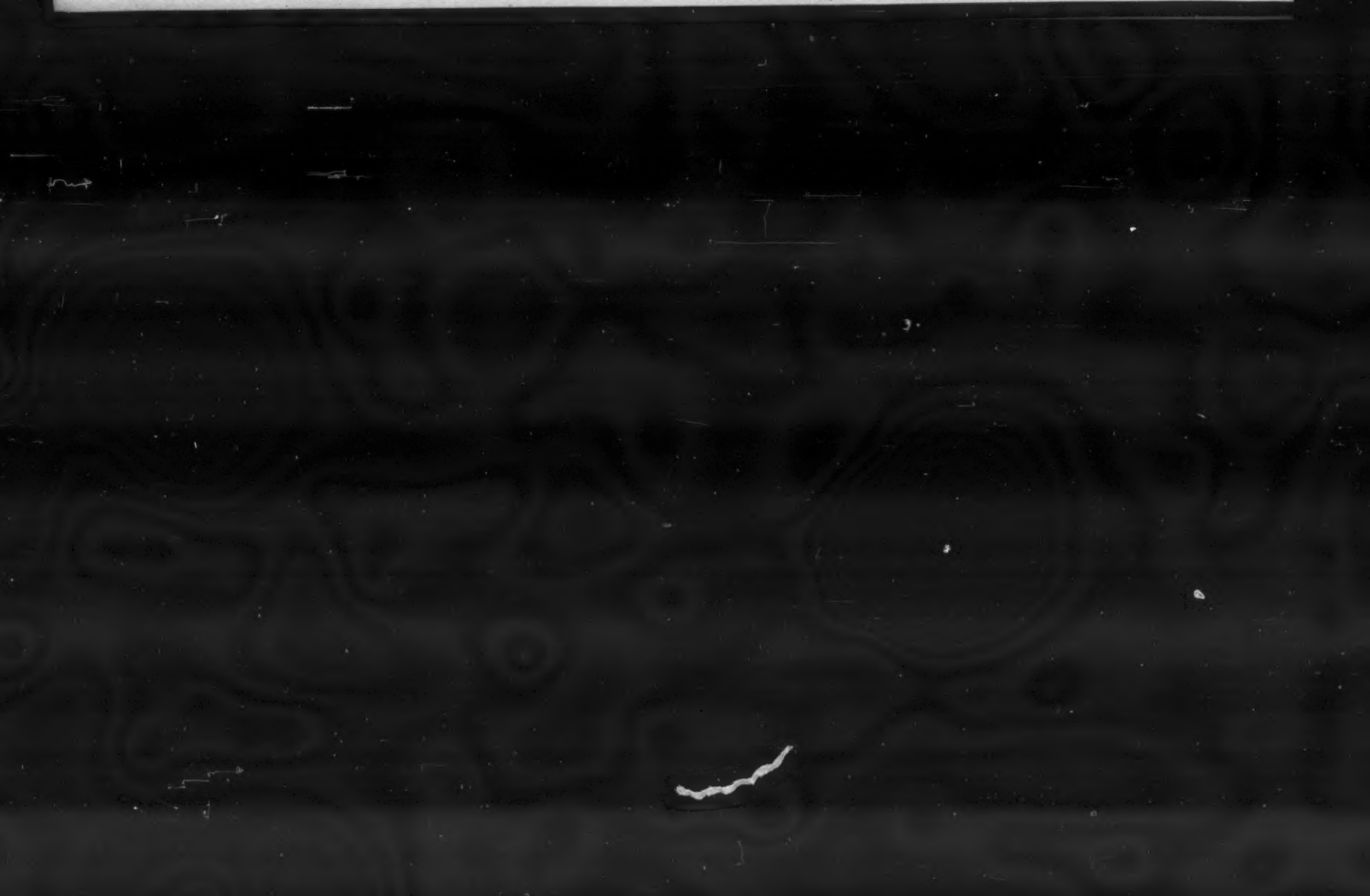
PLATE 201

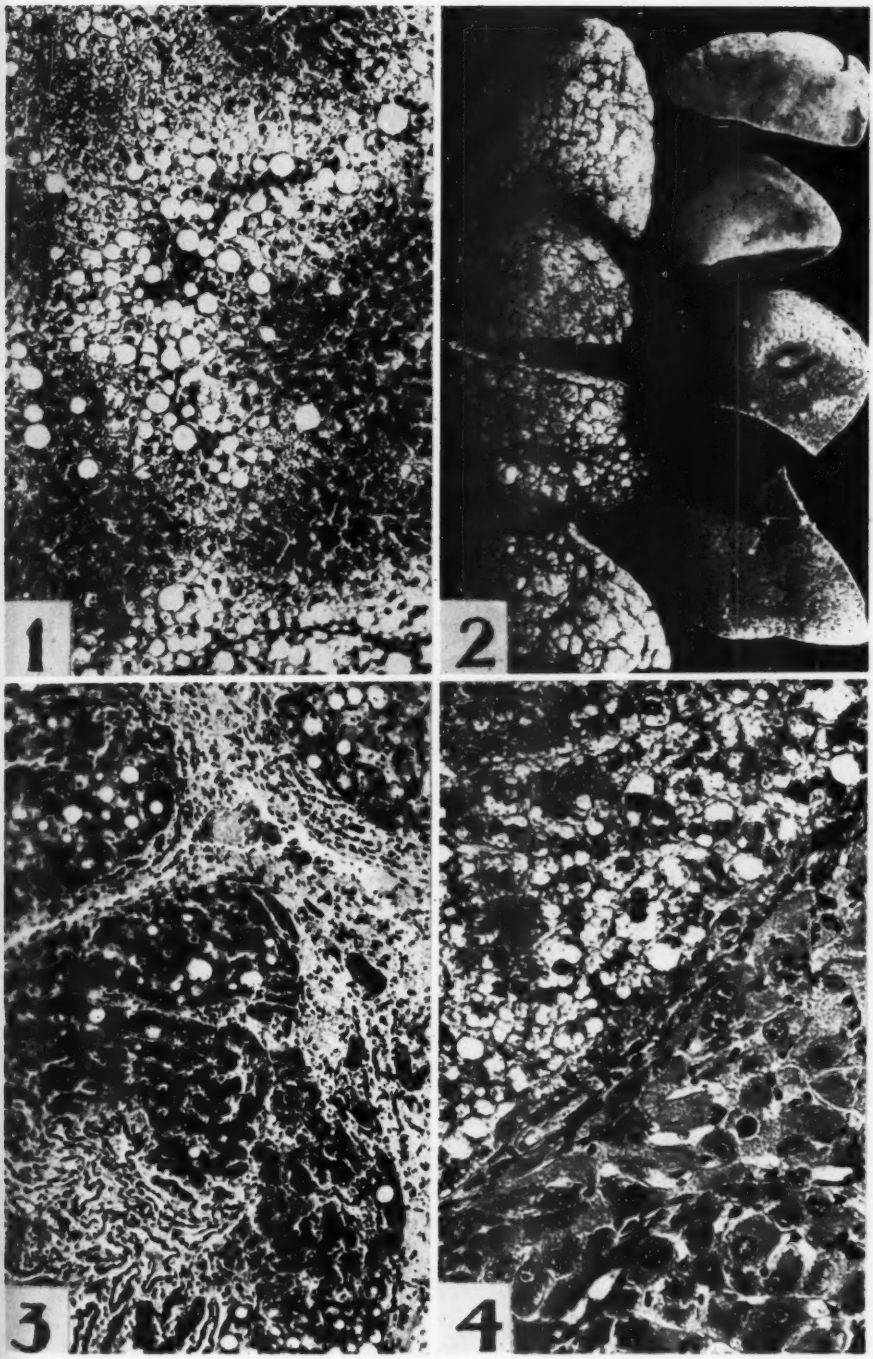
FIG. 1. Zonal distribution of fat and some cellular degeneration and fibrosis in the liver of a rat after 90 days on choline-deficient Diet 43A. Hematoxylin and eosin stain. × 120.

FIG. 2. Variation in the gross appearance of cirrhotic livers from different rats on Diet 43A.

FIG. 3. Extensive perilobular fibrosis and the alteration of sinusoids in the cirrhotic liver of a rat after 8 months on Diet 43A. Hematoxylin and eosin stain. × 145.

FIG. 4. Variation in cell construction and fat content of different lobules of a cirrhotic liver. Hematoxylin and eosin stain. × 300.





Copeland and Salmon Neoplasms in Choline Deficiency

PLATE 202

FIG. 5. Liver of a rat after 10 months on Diet 43A, exhibiting cirrhosis, deposition of an insoluble pigment, and cysts lined by flattened endothelium-like cells. There is a focal loss of parenchymal cells and a proliferation of spindle-shaped cells. Hematoxylin and eosin stain. × 130.

FIG. 6. Area of the liver of a rat showing focal loss of parenchymal cells and a proliferation of endothelium-like cells. Hematoxylin and eosin stain. × 300.

FIG. 7. Hyperplastic nodule on a cirrhotic liver showing a significant increase in parenchymal cells, small in size and peculiar in arrangement. Hematoxylin and eosin stain. × 400.

FIG. 8. Area of liver showing giant cells with a peculiar staining reaction, granular cytoplasm, and a cord-like arrangement of some of the cells. Hematoxylin and eosin stain. × 300.

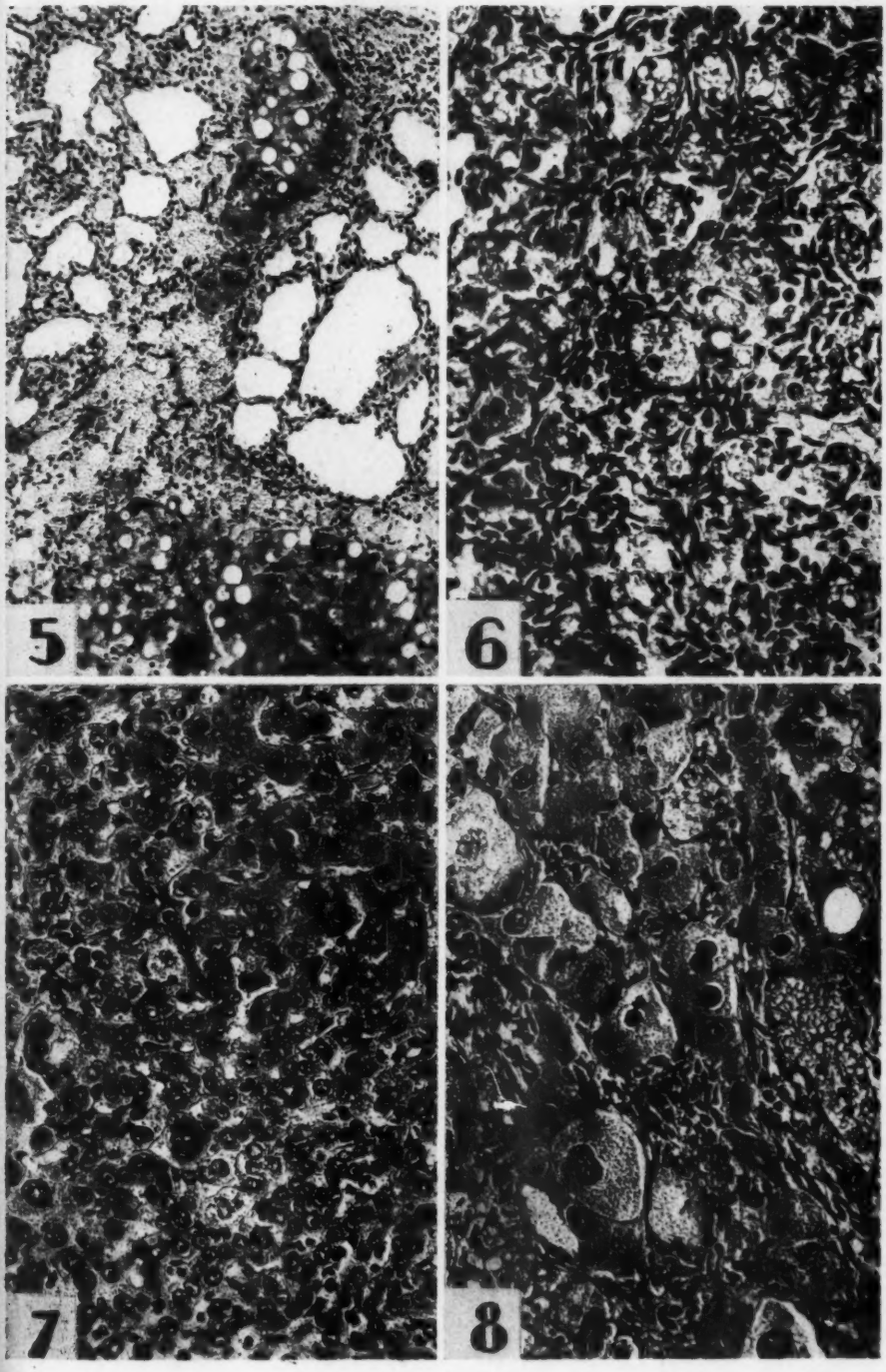

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PLATE 203

FIG. 9. Gross dissection showing the appearance of the liver of an animal on Diet 43A for 10 months or longer. The nodules extend down into the connective tissue of the mesentery.

FIG. 10. Neoplasm of the nature of a hepatoma. Of note are the numerous mitotic figures. Hematoxylin and eosin stain. × 275.

FIG. 11. Definite cell (cytoplasmic) membranes and peculiar arrangement of cells in a hepatoma. Hematoxylin and eosin stain. × 300.

FIG. 12. Cells arranged in a cord-like fashion with wide sinusoid-like spaces between the cords in a hepatoma. Hematoxylin and eosin stain. × 300.

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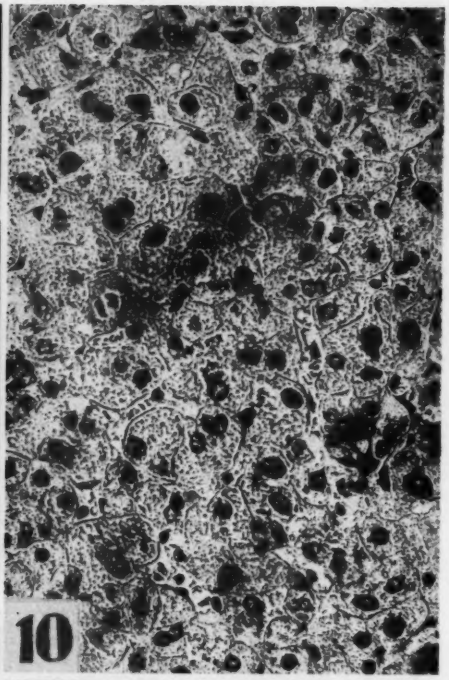
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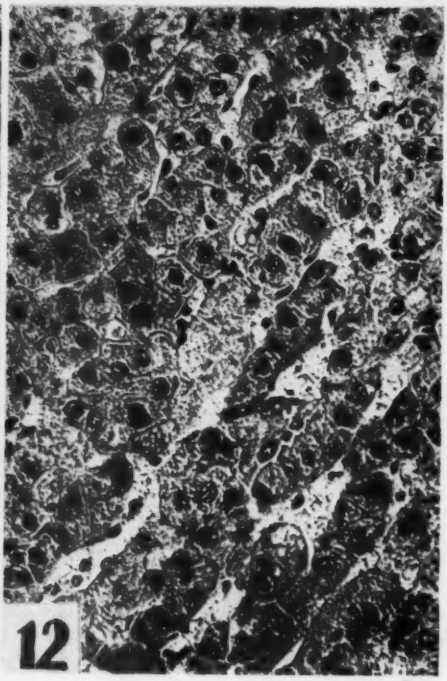
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PLATE 204

FIG. 13. Neoplasm of the nature of an adenocarcinoma from liver-like cells within the hepatic lobule. Hematoxylin and eosin stain. × 300.

FIG. 14. Neoplasm of the nature of an adenocarcinoma in the interlobular connective tissue of the liver. Hematoxylin and eosin stain. × 145.

FIG. 15. An area of more extensive growth of the adenocarcinoma to show the nature and arrangement of the cells and the stroma. Hematoxylin and eosin stain. × 450.

FIG. 16. Neoplasm of the nature of a hemangio-endothelioma showing the vascular nature and the type and arrangement of the cells. Growths of this nature were found in the subcutaneous and retroperitoneal fat. Hematoxylin and eosin stain. × 260.

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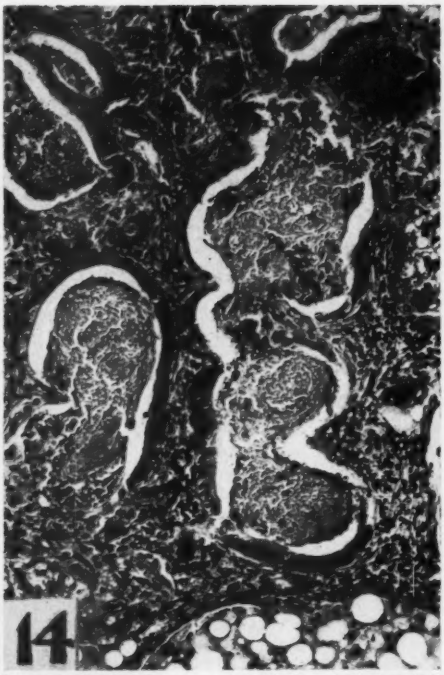
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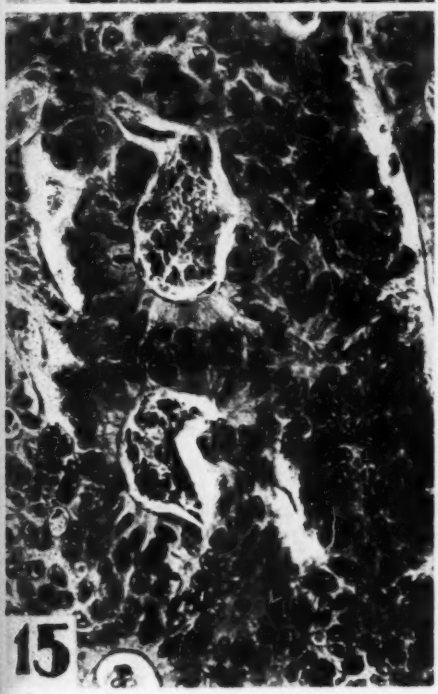
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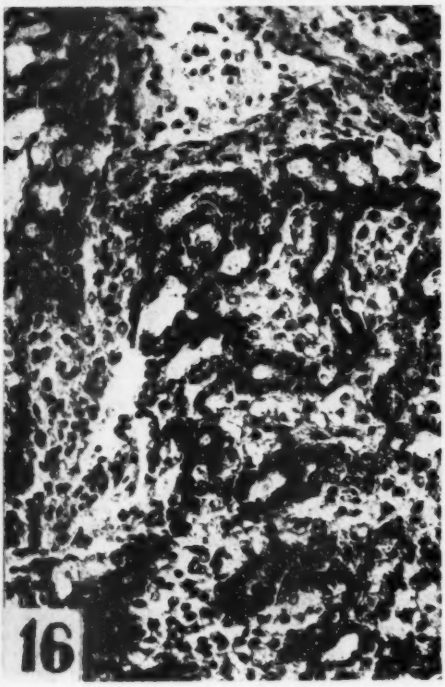
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PLATE 205

FIG. 17. Lung of a rat on Diet 43A for 12 months, exhibiting nodular growths of various sizes.

FIG. 18. Neoplasm of the nature of a primary carcinoma in the lung of a rat. Some of the cells have an adenomatous arrangement. Hematoxylin and eosin stain. × 590.

FIG. 19. Metaplasia of the epithelium lining a bronchiectatic cavity. The epithelium exhibits a transition from simple columnar to typical stratified squamous epithelium. The neoplasm can be seen underlying the epithelium. Hematoxylin and eosin stain. × 300.

FIG. 20. Papillomatous growth from a lung showing acini-like spaces containing sheets of cells. Hematoxylin and eosin stain. × 300.

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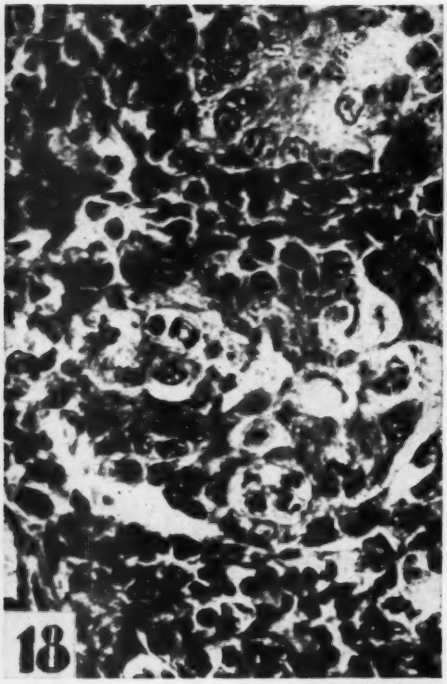
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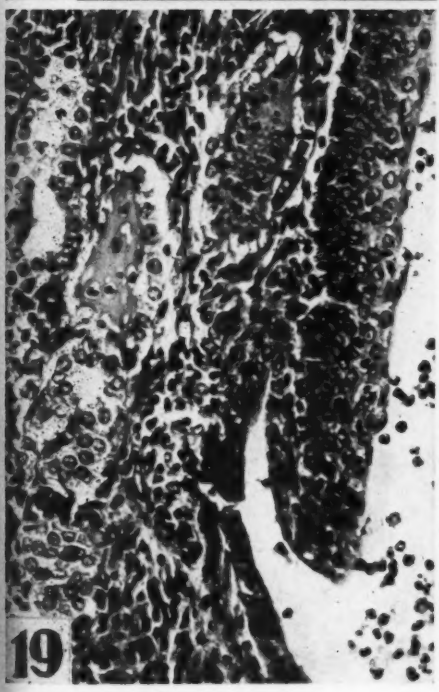
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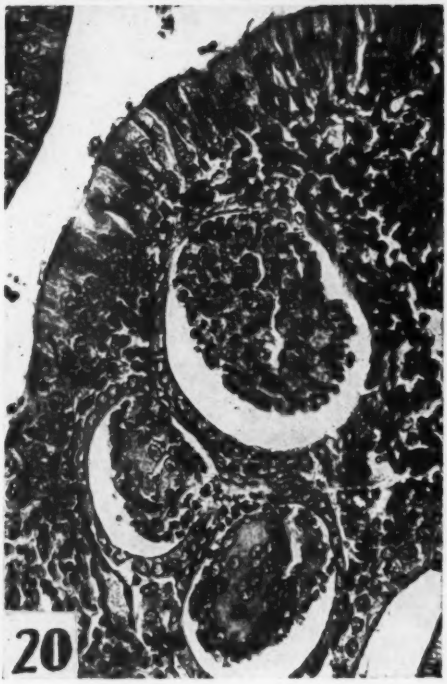
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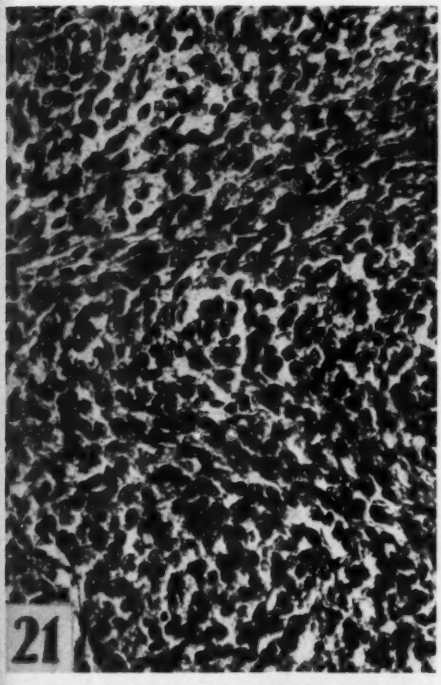
PLATE 206

FIG. 21. Neoplasm of the nature of a sarcoma. This growth was retroperitoneal in location. Of note are the cellular nature of this growth and the relatively small amount of connective tissue fibers present. The cells are arranged in planes and are whorled in different directions. Hematoxylin and eosin stain. × 290.

FIG. 22. Neoplasm of the nature of a fibrosarcoma showing a large amount of fibrous tissue. Hematoxylin and eosin stain. × 290.

FIG. 23. Neoplasm of the nature of a sarcoma showing invasion and replacement of muscle. There are numerous mitotic figures. Hematoxylin and eosin stain. × 470.

FIG. 24. Sarcoma enlarged to show the nature of the cells and an atypical mitotic division figure. Hematoxylin and eosin stain. × 820.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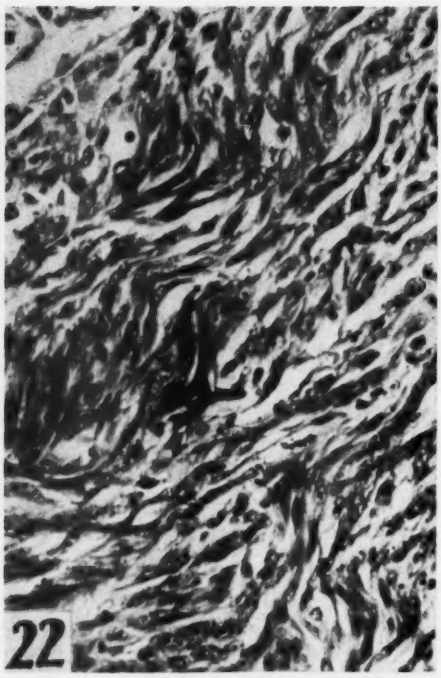
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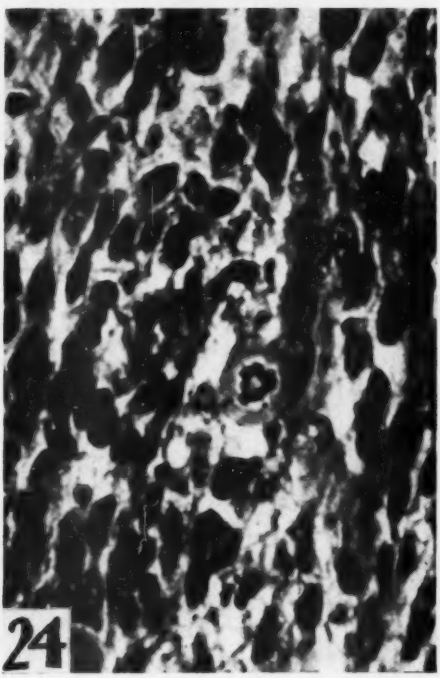
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